FINAL REPORT

Sediment Ecosystem Assessment Protocol (SEAP): An Accurate and Integrated Weight-of-Evidence Based System

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Acronyms

ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
BDL	Below detection limit
DDT	Dichlorodiphenyltrichloroethane
DGT	Diffusive gradient in thin film
DO	Dissolved oxygen
DoD	Department of Defense
EC50	Median effective concentration
EJ	Emergent juvenile
ERDC	Engineer Research and Development Center
ERL	Effects range low
ERM	Effects range median
ESTCP	Environmental Security Technology Certification Program
GIS	Geographical information system
GSI	Groundwater-surface water interface
HDPE	High density polyethylene
HOC	Hydrophobic organic compound
ID	Inner diameter
LC50	Medial lethal concentration
LOEC	Lowest observable effect concentration
NASP	Naval Air Station Pensacola
NBSD	Naval Base San Diego
NCBC	Naval Construction Battalion Center
NESDI	Navy Environmental Sustainability Development to Integration
NOEC	No observable effect concentration
NSA	Naval Support Activity
NTC	Naval Training Center
OCP	Organochlorine pesticide
OD	Outer diameter
ORP	Oxidation-reduction potential
PAH	Polycyclic aromatic hyrdocarbon
PCB	Polychlorinated biphenyl
PDMS	Polydimethylsiloxane
PSD	Passive sampling device
PTFE	Polytetrafluoroethylene
RDX	Cyclotrimethylenetrinitramine
RPM	Revolutions per minute
SEA Ring	Sediment Ecotoxicity Assessment Ring
SEAP	Sediment Ecosystem Assessment Protocol
SED	Surficial sediment toxicity exposure chamber
SERDP	Strategic Environmental Research and Development Program
SPAWAR	Space and Naval Warfare Systems Center
SPME	Solid phase microextraction
SQG	Sediment Quality Guideline
SQO	Sediment Quality Objective
SWI	Sediment-water interface
TDDT	Total dichlorodiphenyltrichloroethane
TIE	Toxicity identification evaluation
TMDL	Total maximum daily load

TNT	Trinitrotoluene
USACE	United States Army Corps of Engineers
USEPA	United States Environmental Protection Agency
UV	Ultra violoet
VOC	Volatile organic compound
WC	Water column
WLR	Weighted logicstic regression
WOE	Weight of evidence

EXECUTIVE SUMMARY

Objective

The purpose of this research was to develop an efficient, accurate and integrated approach for the assessment of ecosystem risk and recovery at sites where contaminated sediments exist, or previously existed. The work addressed the two high priority needs identified in SERDP ERSON-07-01: 1) Develop and evaluate rapid measurement tools/ screening assays to efficiently assess the ecological risk and recovery at contaminated sites for relevant receptors, particularly for assessing natural recovery; and, 2) Assess the ecological impacts to benthic communities of remedial technologies currently in use at contaminated sediment sites.

Summary of Process/Technology

We demonstrated that the development of an integrated system (Sediment Ecosystem Assessment Protocol – SEAP) incorporating rapid *in situ* hydrological, chemical, biological and toxicological measurements provides concise, decision-oriented scientific and ecological information to improve the overall management of contaminated sediment sites. A unique ability to simultaneously assess these interdependent processes is achieved by integrating multiple tools/assays simultaneously to better link exposure and effects measures. This includes placing *in situ* toxicity and bioaccumulation test systems alongside the existing Trident and UltraSeep Systems via the Sediment Ecotoxicity Assessment (SEA) Ring system. The Trident is a multi-sensor sediment probe device that is designed to rapidly identify groundwater-surface water (GSI) discharge zones, and to sample pore water from these areas. The UltraSeep System provides the ability to directly and continuously quantify GSI discharge rates and collect flow proportional samples to quantify both water and chemical flux. The SEA Ring system allows for multiple species of ecologically relevant organisms to be deployed in 3 different exposures (overlying water, sediment-water interface, and bulk sediment) for approximately 2 day periods. Two days is the chosen period of exposure for the following reasons: longer exposures can be stressful to some caged organisms, this period of time has been shown in previous research to be as sensitive as 10 d USEPA laboratory-based sediment toxicity assay results; the SEAP method is meant to provide a relative ranking of station risk identifying high risk sediments in particular (marginal contamination is not the focus) which will often be detected in short exposures; short-term exposures were found adequate for detecting bioaccumulation (not equilibration); and resource requirements are also a consideration, as longer deployments are more costly and subject to failure. In addition, passive sampling devices for detecting nonpolar organics (SPMEs) and metals (DGTs) are also provided to detect exposures in overlying waters and through near-surface sediment depth profiles. The SEA Ring allowed for measurements of multiple endpoints, ranging from mortality to sublethal effects (e.g., feeding, tissue uptake, embryo development). The SEA Ring proved quite versatile, and was deployed in a wide range of habitats and conditions ranging from Pacific to the Gulf of Mexico, one to ten meter depths (diverless), oligotrophic to eutrophic, cool to warm, and large to fine grained sediments. The data from the simultaneous exposure and effects measures over a wide range of contamination gradients provided accurate, short-term measures for ecological risk characterizations. These data were incorporated into a GIS Weight-of-Evidence, Weighted Logistic Regression approach that statistically linked site physical and chemical stressors with adverse biological responses. This approach allows site managers to quickly and accurately determine areas of highest risk along with the stressors which may require remediation.

Background

Traditional approaches for addressing contaminated sediments have focused on relatively crude measurements to characterize chemical contamination of bulk sediments. Effective risk management and remedy selection decisions are unlikely without first characterizing the pathways and compartments responsible for contaminant exposures. In addition to the fate and transport of migrating sediment and groundwater contaminants, bioresponses from surficial sediments, upwelling groundwater, and sediment

pore-water contaminants, from mobilization of sediment-bound contaminants, and from overlying surface waters need to be assessed concurrently. Coupling a suite of laboratory and field physical, chemical, and toxicity screening tools and assays to current Trident and UltraSeep systems will lead to improved characterization of contaminant exposure and receptor effect linkages. Data from these multiple lines-of-evidence then can be integrated into a weight-of-evidence-based geographic information system (WoE-GIS), providing statistically based rankings of a site's likely dominant physical and chemical stressors.

The three Tasks of research involved:

1. Review and selection of bioassays (Year 1)
2. Selection of bioassays and biomimetic assays for field deployments. Integration with the UltraSeep and Trident units (Year 2)
3. Field tests of SEAP units and a weight-of-evidence study (Year 3).

Benefits

This project lays the foundation for certification of methods and development of a conceptual framework and user's guide for improving the overall management of contaminated sediment sites. The screening assay approach enables assessment of many stations within a short time frame, and a straightforward, quantitatively based weight-of-evidence approach graphically demonstrates spatial and temporal displays of sediment quality and dominant stressor relationships with ecological risk. This integrated approach is expected to be useful not only for specific groundwater applications, but also for reducing the uncertainty of the risk assessment process by improving the linkage between chemical exposure and adverse biological responses, thus providing an improved decision making process.

Transition Plan

Future research will be conducted if funded by ESTCP and include optimization of all aspects of the SEA Ring, including diverless deployment. This work was performed in collaboration with site managers at coastal military installations. Results are being transitioned through publication of peer-reviewed journal articles, technical reports, and technical symposia. It is anticipated that this work lays the foundation for certification of methods and development of a conceptual framework and user's guide for site managers under follow-on support from the Environmental Security Technology Certification Program (proposal under review). A patent application has been filed (U.S. Navy and University of Michigan) for the SEA Ring technology.

Conclusions

The key accomplishments and conclusions for Year 1 and Task 1 were as follows:

1. Bioassay performance criteria were developed for selection of optimal short listed bioassays, as follows:
   - Developmental status
   - Availability
   - Robustness/Relevance
   - Adaptability to in situ
   - Exposure duration
   - Test volume
   - Salinity tolerance
   - Contaminant sensitivity
   - Confounding effects
   - Costs
2. Short listed bioassays for lab screening are:

- QwikLite (Dinoflagellate, *Pyrocystis lunula*) luminescence – 24 h
- QwikLite (Dinoflagellate, *Ceratorcorys horrida*) luminescence – 24 h
- Rotifer *(Brachionus plicatilis*) survival – 24-48 h
- Mussel (*Mytilus galloprovincialis*) embryo-larval development – 48 h
- Sea urchin fertilization – 1 h

3. Short listed bioassays for field screening are:
   - QwikLite (Dinoflagellate, *Pyrocystis lunula*) luminescence – 24 h
   - Rotifer *(Brachionus plicatilis*) survival – 24-48 h
   - Mussel (*Mytilus galloprovincialis*) embryo-larval development – 48 h
   - Amphipod (*Eohaustorius estuarius*) survival – 2-10 d
   - Amphipod (*Leptocheirus plumulosus*) survival – 2-10 d
   - Mysid (*Americamysis bahia*) survival – 48-96 h
   - Polychaete (*Neanthes arenaceodentata*) feeding rate – 48 h
4. As was expected, responses to different salinity and temperature combinations on the toxicity of copper differed among the different test endpoints. In general, however, toxicity was greatest at the lowest test salinities, which might be linked to increased uptake of copper as a consequence of speciation and/or competition for binding sites. Intolerance to certain salinities and temperatures, and the effects of salinity and temperature on metal bioavailability should be taken into account when developing an approach to *in situ* testing.
5. With one exception, all toxicity tests were deemed successful, based on performance in the negative controls. Most test endpoints ranged from 80 to close to 100% in the controls. *L. plumulosus* survival was quite variable upon examination after 10 days of exposure, however, so only data for 2 and 4 day exposures for this species were deemed acceptable.
6. Overall, pore water samples resulted in moderate to low toxicity. QwikLite tests ranked relatively high in terms of sensitivity. *C. horrida* was the most sensitive test, but it also was more susceptible to ammonia toxicity. *P. lunula* is not nearly as sensitive to ammonia, removing this potential confounding factor from all samples tested. *P. lunula* was approximately equally as sensitive as standard test methods such as sea urchin fertilization and mussel embryo larval development. It should be noted, however, that conducting the QwikLite test with *P. lunula* is much more cost effective and simpler than the other two tests. Mysids were generally unimpacted in all samples after 2 days of exposure, but sensitivity increased over time, resulting in an average ranking after 7 days similar to that for QwikLite (*P. lunula*), sea urchin fertilization, and mussel embryo-larval development. Neither species of amphipod was particularly negatively impacted by the samples tested. The feeding rate assay was overall less sensitive than most other tests, but responded similarly in terms of relative sensitivity to the other species. The rotifer, while comparably sensitive overall (after 2 days of exposure), did not agree with responses for the other test organisms.
7. Various chamber designs were evaluated for in situ testing of mussel embryos. The scintillation and shell vials proved to be optimal. Salinity within the vials rapidly increased during the first few hours of the exposure. Within 4 hours, salinity in the vials was 79 and 88% of the salinity in the external environment for static and flow through conditions, respectively. Steady-state conditions were achieved within 6 hours under the flow conditions, and by the 18 hour time-point under static conditions.
8. The sensitivity of the candidate bioassays to currents was evaluated with shaker experiments and large and small chambers with two mesh sizes. Amphipods and mysids both faired quite well in the Large chambers at 100 RPM after 48 h, but had reduced survival (at 48 h only) at 150 RPM. The Small chambers resulted in relatively little impacts on survival for both species at 100 RPM,

but survival for both species was reduced at 150 RPM. The amphipods are more tolerant of higher degrees of physical stress. Both amphipods and mysids had high survival rates in the 48 h field deployments off the SPAWAR Pier in both chamber sizes.

9. A novel sublethal feeding bioassay was conducted with the polychaete, *Neanthes*. The utility of a growth endpoint in field exposures may be problematic due to the differences in food quality and quantity at different sites and the fact that feeding specified rations to field organisms (as is done in laboratory testing) might be logistically challenging. In addition, significant growth requires relatively long exposure times for polychaetes.

The key accomplishments and conclusions for year 2 and Task 2 were as follows:

1. Test sites were selected and field deployments were successfully completed in San Diego and Pensacola.
2. Further performance evaluation criteria of the bioassays were evaluated with field deployments and laboratory testing.
3. The field bioassays and biomimetic assays were selected and incorporated into a new exposure platform for deployments adjacent to the UltraSeep platform, or at sites identified by the Trident probe and historical data indicating that they were potentially contaminated. The SEA Ring platform was designed and successfully deployed.
4. The system was adjusted and reevaluated on-site.

The key accomplishments and conclusions for year 3 and Task 3 were:

1. The SEA Ring platform was successfully deployed in San Diego Bay (twice at different areas) and in Pensacola Bay in areas with varying contaminant types and sediment characteristics.
2. The Sediment Ecotoxicity Assessment (SEA) Ring platform allowed for simultaneous deployment of multiple species exposed via three compartments (overlying water, sediment water interface, and bulk sediment), along with passive samplers (diffusion gradients in thin films (DGTs) and solid phase microextraction (SPME) fibers for metal and non-polar organic sorption, respectively. The SEA Ring also has water quality sensors installed to continuously monitor water quality parameters from within the in situ exposure cages.
3. Initial surveys with the Trident probe sampled pore waters that were tested in 24 hr (or less) bioassays as the screening evaluation to determine optimal siting of the SEA Rings the following day. The SEA Rings (with test organisms) were deployed for 48 hr, and on some deployments bioaccumulation testing with both organisms and SPMEs continued for several days afterwards.
4. Results at all three sites were useful at identifying "hot" spots of toxicity with relationships to chemical contaminant levels. Short-term bioaccumulation testing with SPMEs showed strong correlations with tissue concentrations, but not bulk sediment concentrations.
5. The SEA Ring was modified with a water pump system to ensure low dissolved oxygen concentrations did not occur within the exposure cages.
6. A weight-of-evidence based GIS approach based on logistic regressions allowed for a statistical ranking of stressor-effect relationships. Recently, the SEA Ring has been modified so that it can be deployed without divers into harbor waters of up to 40 ft. in depth.

The conclusions from the three field deployments showed the integration of various endpoints and measures was useful in characterizing the test sites investigated. Toxicity, bioaccumulation, bulk chemistry, and bioavailability as deemed by pore water concentrations derived from uptake by passive

samplers followed the expected gradient at NBSD, and suggested hydrophobic organics (i.e. PAHs) might be important stressors, while bulk metals and DGT concentrations appeared to be of less concern. At NAS Pensacola, similar results were observed. However, the Trident and UltraSeep were used to evaluate the potential for groundwater-surface water interactions to be contributing to historically defined effects at the southern end of the water body. Although groundwater was discharging into the surficial sediments, analysis of flow-weighted samples of the discharge revealed little to no chemical contamination associated with the infiltrating groundwater. Bulk chemistry, toxicity, and bioaccumulation, however, pointed to possible PAH-associated toxicity, which could have been exacerbated by UV photoinduced toxicity, explaining the difference between *in situ* and laboratory data for the shallow site. The importance of continuous water quality sensing was very clear at the Chollas Creek site, where diurnal drops in dissolved oxygen may have contributed to amphipod toxicity. That site, however, appears to be improving based on lower bulk chemical concentrations and toxicity than previously observed. This could be associated with recent restoration efforts upstream and reduced inputs of organophosphate pesticides, but the potential for temporal and spatial variability of results was noted.

The GIS WOE/WLR showed that a stressor-response hypotheses generated by the spatial analyses can provide insight into the process(es) influencing local recovery or degradation, and provide guidance for corresponding remedial strategies if deemed necessary. Despite uncertainties in the study of San Diego Bay, overall the application of a WOE/WLR-based ecological assessment to benthic survey and *in situ* toxicity field data for the Naval Station study area was successfully applied to SERDP project data for the San Diego harbor study area, and effectively delineated screening-level stressor hypotheses for use in site management. The study results indicated that ecological risk and associated remediation strategies in the harbor would be best focused on the Chollas and Paleta Creek areas, as the dock area of the inner harbor sampled during the 2008 SERDP study had comparatively lower levels of risk. For areas with predicted ecological risk, pesticide exposure (represented as cumulative pesticide exposure) generally provided the greatest increase in ecological risk, pointing to this stressor source as remediation priority. This study showed potential for the application of this type of spatial analysis approach to other harbor-based study areas, particularly those with greater data variability (i.e., more abundant and severe instances of biological impact) and a sampling design limiting geographic sampling bias further.

The SEAP method is unique. No other in situ systems exist that provide simultaneous exposures to multiple species in multiple exposure compartments, and allow for co-existing collection of chemical data from each exposure compartment. These SEAP characteristics are its primary advantage over existing methods. Existing methods can be divided into laboratory and in situ based approaches, each of which have their own unique strengths and limitations (Adams et al., 2005; Burton et al., 2005). The limitations of the SEAP approach are that it is non-standardized, requires specialized construction, may require a diver in deep waters, and is subject to vandalism and weather disruption.

In summary, the project met the stated objective to develop an efficient, accurate and integrated approach for the assessment of ecosystem risk and recovery at sites where contaminated sediments exist, or previously existed. We demonstrated that the development of an integrated system (Sediment Ecosystem Assessment Protocol – SEAP) incorporating rapid *in situ* hydrological, chemical, biological and toxicological measurements provides concise, decision-oriented scientific and ecological information to improve the overall management of contaminated sediment sites. A unique ability to simultaneously assess these interdependent processes is achieved by integrating multiple tools/assays simultaneously to better link exposure and effects measures. This includes placing *in situ* toxicity and bioaccumulation test systems alongside the existing Trident and UltraSeep Systems via the Sediment Ecotoxicity Assessment (SEA) Ring system. The SEA Ring system allows for multiple species of ecologically relevant organisms to be deployed in 3 different exposures (overlying water, sediment-water interface, and bulk sediment) for approximately 2 day periods. In addition, passive sampling devices for detecting nonpolar organics (SPMEs) and metals (DGTs) are also provided to detect exposures in overlying waters and through near-surface sediment depth profiles. The SEA Ring allowed for measurements of multiple endpoints, ranging

from mortality to sublethal effects (e.g., feeding, tissue uptake, embryo development). The SEA Ring proved quite versatile, and was deployed in a wide range of habitats and conditions ranging from Pacific to the Gulf of Mexico, one to ten meter depths (diverless), oligotrophic to eutrophic, cool to warm, and large to fine grained sediments. The data from the simultaneous exposure and effects measures over a wide range of contamination gradients provided accurate, short-term measures for ecological risk characterizations. These data were incorporated into a GIS Weight-of-Evidence, Weighted Logistic Regression approach that statistically linked site physical and chemical stressors with adverse biological responses. This approach allows site managers to quickly and accurately determine areas of highest risk along with the stressors which may require remediation.

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1.0 LITERATURE REVIEW SUMMARY

An extensive literature review was conducted to determine the state of the science with respect to *in situ* bioassays and determine which tests would be most relevant and promising in the context of this research. The literature review covered the advantages and limitations of in situ bioassays, provided suggestions for appropriate chamber and experimental design, and discusses case histories. The focus of the literature review was on estuarine/marine organisms, but freshwater studies were also included where information was lacking for marine systems, or where the freshwater data appeared to be particularly valuable. The literature review formed the basis for deriving our 'short list' of bioassays for this project. Previously defined evaluation criteria were taken into account during the literature review research, and in decision making with respect to the 'short list'. The literature review was published as a SPAWAR technical report (Rosen et al. 2009). The basis for making the decisions to use various tests and the outcome of those decisions are summarized below.

Performance criteria that were used to assess test method relevance included:

- Developmental status
- Availability
- Robustness/Relevance
- Adaptability to in situ
- Exposure duration
- Test volume
- Salinity tolerance
- Contaminant sensitivity
- Confounding effects
- Costs

A basic ranking system was used as a means of quantifying the practicality of using standardized tests as screening tools, either in short-term laboratory or *in situ* exposures (Table 1-1). Tests were ranked using the above performance criteria. The ranking was based on a number system with "1" indicating a low or poor ranking, and 3" indicating a good or high ranking. When inadequate data were available, a best professional judgment call was made, as indicated in bold.

Reference

Rosen G, Chadwick DB, Poucher SL, Greenberg MS, Burton GA. 2009. *In Situ* Estuarine and Marine Toxicity Testing: A Review, Including Recommendations for Future Use in Ecological Risk Assessment. Space and Naval Warfare Systems Center Pacific (SSC Pac) Technical Report 1986. September 2009. 73pp.

Table 1-1. Ranking of key estuarine/marine toxicity test methods that could be appropriate for short term laboratory or in situ screening, based on project-identified performance criteria.

Test Endpoint	Lab or Field?	Developmental Status	Availability	Robustness/Relevance	Test volume	Exposure Duration	Salinity Tolerance	Temperature Tolerance	Contaminant Sensitivity	Confounding Effects	Costs	Total
Mysid (*A. bahia*) Survival	L, F	3	3	3	3	2	3	3	3	3	2	28
Rotifer (*B. plicatilis*) Survival	L, F	3	3	**2**	3	3	3	3	2	3	1	26
Amphipod (*E. estuarius*) Survival	L, F	3	3	3	3	1	3	2	2	3	2	25
Amphipod (*L. plumulosus*) Survival	L, F	3	3	3	3	1	3	2	2	3	2	25
Mussel (*Mytilus sp.*) Embryo Development	L, F	3	2	3	3	2	2	2	3	2	2	24
Polychaete (*N. arenaceodentata*) Feeding Rate	L, F	**2**	3	3	3	3	2	2	2	**2**	2	24
Dinoflagellate (*P. lunula*) Luminescence	L,F	**2**	3	**2**	3	3	2	2	**3**	3	1	24
Oyster (*C. gigas*) Embryo Development	L, F	3	1	3	3	2	2	2	3	2	2	23
Amphipod (*R. abroniu*s) Survival	L, F	3	3	3	**3**	1	2	2	2	2	2	23
Amphipod (*A. abdita*) Survival	L, F	3	3	3	**3**	1	2	2	2	2	2	23
Polychaete (*N. arenaceodentata*) Survival, Growth	L, F	3	3	3	3	1	2	2	2	3	1	23
Dinoflagellate (*C. horrida*) Luminescence	L,F	**2**	3	**2**	3	3	2	2	**3**	2	1	23
Sea urchin (*S. purpuratus*) Fertilization Success	L	3	2	2	3	3	1	1	2	3	2	22
Sea urchin (*S. purpuratus*) Embryo Development	L, F	3	2	3	3	2	1	1	3	1	2	21
Bacterium (*V. fischeri*) Luminescence	L	3	3	1	3	3	1	1	1	2	1	19

1 = Low Ranking (poor), 3 = High Ranking (good)
Bold = lack of knowledge

2.0 SHORT LIST TEST SPECIES SELECTION

One of the results of the literature review was the ability to down select towards a core group of tests that would have the greatest chance of success with project goals, which was assessed using the previously identified test performance criteria (Section 1) and literature review (Table 2-1). Some of these toxicity tests are likely to be more appropriate for laboratory screening, while others are well suited for field screening. There is quite a bit of overlap, however, with some tests being potentially useful in both settings. Table 1-1 was used to a great degree to identify the short list below. It should be noted, however, that in at least one case, a test was placed on the short list even though its overall ranking was relatively low. This was because one or more of the performance criteria outweighed the overall ranking process. For example, although sea urchin fertilization tests can only be conducted in the laboratory and scored low for salinity tolerance, temperature tolerance, and availability, the test's high developmental status (i.e. extensive use in regulatory programs, including those leveraged with this project) low test volume, and short duration were deemed sufficient to include on our short list. The short list is summarized below:

Lab Screening

- QwikLite (Dinoflagellate, *Pyrocystis lunula*) luminescence – 24 h
- QwikLite (Dinoflagellate, *Ceratorcorys horrida*) luminescence – 24 h
- Rotifer *(Brachionus plicatilis*) survival – 24-48 h
- Mussel (*Mytilus galloprovincialis*) embryo-larval development – 48 h
- Sea urchin fertilization – 1 h

Field Screening

- QwikLite (Dinoflagellate, *Pyrocystis lunula*) luminescence – 24 h
- Rotifer *(Brachionus plicatilis*) survival – 24-48 h
- Mussel (*Mytilus galloprovincialis*) embryo-larval development – 48 h
- Amphipod (*Eohaustorius estuarius*) survival – 2-10 d
- Amphipod (*Leptocheirus plumulosus*) survival – 2-10 d
- Mysid (*Americamysis bahia*) survival – 48-96 h
- Polycheate (*Neanthes arenaceodentata*) post exposure feeding rate – 48 h

Table 2-1. Examples of estuarine/marine test endpoints that have been successfully reported in the literature.

Organism Type	Species	Endpoint(s)	Exposure Duration (d)	Reference
Mussel	*Mytilus galloprovincialis*	Embryo-larval development	2 2 2	Anderson et al. (1998) Geffard et al. (2001) Katz and Rosen (2005)
Oyster	*Crassostrea gigas*	Embryo-larval development	2	Geffard et al. (2001)
Sea Urchin	*Paracentrotus lividus*[2]	Embryo-larval development and	3	Beiras et al. (2001)
Sea urchin[1]	*Strongylocentrotus purpuratus*	Embryo-larval development	3 to 4	Anderson et al. (1996, 2001)
Mussel, Clam	*Mytilus edulis, Mytilus galloprovincialis, Macoma nasuta, Macoma balthica*	Bioaccumulation, growth	28 to 90	ASTM (2003), Salazar and Salazar (2007)
Clam	*Mercenaria mercenaria*	Growth	7	Ringwood and Keppler (2002)
Amphipod	*Eohaustorius estuarius*	Survival	10	Anderson et al. (2004)
Amphipod	*Corophium volutator*[2]	Survival	10	Kater et al. (2001)
Polychaete	*Hediste diversicolor*[2]	Survival, post exposure feeding	2 d + 1 hr feeding	Moreira et al. (2005)
Mysid shrimp	*Americamysis bahia*	Survival	0.5 to 3 7	Clark et al. (1986, 1987) Comeleo et al. (1990) Comeleo et al. (1991)
Crab	*Cancer maenus*[2]	Survival, post exposure feeding		Moreira et al. (2006)
Fish	*Cyprinodon variegatus*		5	Clark et al. (1986, 1987)
Fish	*Atherinops affinis*	Bioaccumulation Embryo hatching	28	Richter (2002) Jelinski and Anderson (1996)
Fish	*Menidia berylllina*	Embryo hatching		Jelinski and Anderson (1996)

[1] Laboratory sediment-water interface tests using intact sediment cores.

[2] European species

3.0 EFFECTS OF TEMPERATURE AND SALINITY ON TOXICITY TO SELECT ORGANISMS

A series of laboratory-based experiments were conducted on relevant short list test species and toxicity endpoints to assess the effects that variations in both temperature and salinity might have on these organisms, as these properties aren't controllable in the field (*in situ* deployments). These experiments also included exposures to a model toxicant at the various temperature and salinity combinations. Copper was selected as the model toxicant due to its DoD relevance as a common contaminant of concern and the vast quantities of data available for this chemical for which to make comparisons. Testing was conducted on endpoints including amphipod (*Eohaustorius estuarius, Leptocheirus plumulsosus*) survival, mussel (*Mytilus galloprovincialis*) embryo-larval development, bioluminescence reduction from the dinoflagellate *Pyrocystis lunula* using the QwikLite test system, post exposure feeding rate of the polychaete *Neanthes arenaceodentata,* and rotifer (*Brachionus plicatilis*) survival.

Summarizations of the various temperature, salinity, and copper combinations used in this study are shown in Table 3-1. Temperature and salinity combinations were generally selected based on the standard test method conditions as a starting point and then adjusting upward and downward by an increment that was test method-specific. Copper concentrations were based on previously reported data from our laboratories or the literature, or were determined in multi-concentration exposures in the absence of such data. Physiological tolerances based on the literature review were also considered in the decision, as it was deemed inappropriate to test under conditions that are clearly unacceptable for the test species.

Table 3-1. Summary of different salinity, temperature, and copper combinations used in toxicity tests. A total of 9 salinity and temperature combinations were exposed to clean seawater and at least 1 copper concentration. Bold values indicate those that are closest to standard recommended test condition for species.

Test Species/Endpoint	Salinity (‰)	Temperature (°C)	Copper (µg L^{-1})
Amphipod (*Eohaustorius estuarius*) survival	10, **20**, 30	10, **15,** 20	30,000
Amphipod (*Leptocheirus plumulosus*) survival	10, **20**, 30	15, **20**, 25	800
Mussel (*Mytilus galloprovincialis*) embryo-larval development	25, **30**, 35	10, **15**, 20	1.9-30
Dinoflagellate (*Pyrocystis lunula*) bioluminescence	15, 25, **35**	15, **22**, 30	125
Polychaete (*Neanthes arenaceodenatata*) post-exposure feeding rate	20, 30, **35**	15, **20**, 25	40

3.1. Methods

Test solutions were made from clean seawater diluted appropriately with E-Pure (18 Ω-ohm) to the appropriate salinity. Clean seawater consisted of filtered (0.45 µm) natural seawater pumped into the SPAWAR lab (amphipod, polychaete, mussel) or synthetic sea salts mixed with deionized water (QwikLite). Natural seawater was collected on the incoming high tide near the mouth of San Diego Bay. Amphipod and polychaete testing occurred in 400 mL glass beakers filled with 250 mL of test solution, with 10 animals per replicate. Most tests involved 3 replicates per concentration. Exposure chambers were renewed with fresh solutions at 48 h for 96 h tests. Water quality (temperature, pH, dissolved oxygen, salinity) was measured daily and was within acceptable limits for all tests. Mortalities were assessed and removed daily during the amphipod and polychaete tests. The amphipod endpoint was survival, while the polychaete assay's endpoint was post-exposure feeding rate.

Mussel embryo-larval development tests generally followed standard methods for whole effluent toxicity testing (USEPA 1995), with tests commencing within 4 h of fertilization. Experimental chambers for the embryo tests were 20 ml seawater leached glass scintillation vials filled with 10 ml of test solution and contained approximately 200 embryos each. The endpoint for the mussel tests was normal survival, a combined endpoint of the total number of normally developed (proddisoconch I stage, D-shaped) surviving larvae relative to the number of initial embryos added. Dinoflagellate exposures generally followed the current user guide for the QwikLite test (Assure Controls 2007), with an endpoint of total bioluminescence output from mechanical stimulation during the peak dark phase of their light cycle, which was also expressed relative to the control in some cases..

3.2. *Eohaustorius estuarius* Copper LC50 Confirmation

Limited published data were available for *E. estuarius* sensitivity to copper. Therefore, a multi-concentration test was conducted for this species. Nominal copper concentrations ranged from 3.125 to 50 mg/L. The experiment was conducted over a 96 h period at a temperature of 15 °C and salinity of 20 ‰, the standard test conditions for this test species (USEPA 1994). The experiment resulted in a nominal LC50 of 30.3 mg/L (Figure 3-1). All concentrations resulted in a copper precipitate, which likely reduced the exposure of bioavailable copper to the amphipods. Dissolved concentrations are currently being determined. The LC50 reported here is consistent with published findings of 12.5-25 mg/L (McPherson and Chapman 2000) and 33.3 mg/L (Anderson et al. 2007), both of which are also based on nominal concentrations. McPherson and Chapman (2002) reported a dissolved EC50 of 3.7 mg/L Cu, still indicating relatively low sensitivity of this species to copper.

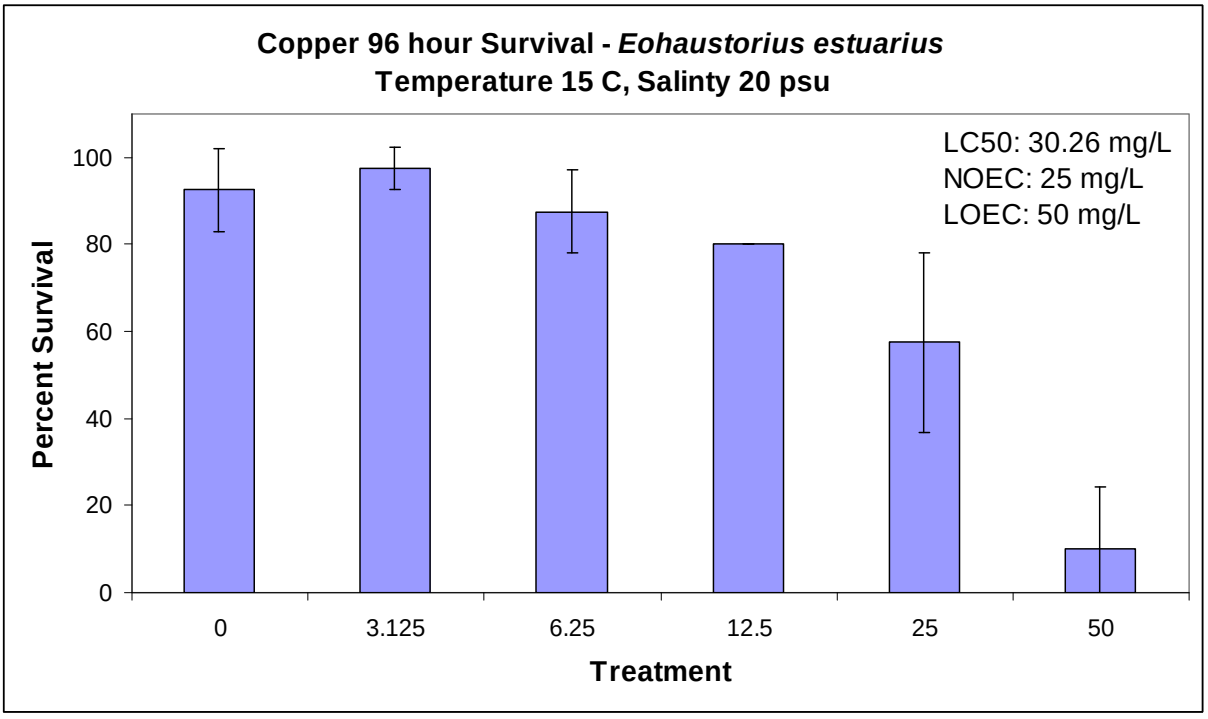

Figure 3-1. Results from copper range-finding experiment for estuarine amphipod *Eohaustorius estuarius.*

3.3. *E. estuarius* Salinity/Temperature Experiment

E. estuarius demonstrated its wide tolerance of varying temperature and salinity conditions with 90% or greater survival under all combinations in the absence of copper (Figure 3-2). The responses to copper

under the different combinations, however, varied substantially. ANOVA indicated significant ($p \leq 0.01$) interactions between copper and temperature ($p=0.01$) as well as copper and salinity ($p<0.0001$), with survival generally lowest with increasing temperature (particularly for 20 °C) and decreasing salinity (survival was particularly low at 10 ‰).

Increasing temperature has been previously reported to increase metal (chromium) toxicity to other amphipod species (*Corophium volutator*), in addition to the mollusk (*Macoma balthica*) and polychaete (*Hediste diversicolor*) (Bryant et al. 1984). The findings with *E. estuarius* are also consistent with reports of zinc toxicity to both the estuarine amphipod (*Corophium volutator*) and the bivalve (*Macoma balthica*) where survival time was lowest at high temperature and low salinity (Bryant et al. 1985). Ozoh (1992) also reported increased accumulation and toxicity of copper with increasing temperature and decreasing salinity to *H. diversicolor*, but cited osmoregulatory and thermal stresses as causes of copper toxicity. The author suggested that under the stress, copper could not be depurated readily in low salinity. It has been suggested that the gill is expected to be a major site of toxic action for copper in estuarine crustaceans (Bianchini et al. 2004). The relatively low survival at lower salinity observed for *E. estuarius* might be linked to increased uptake of copper at the gill as a consequence of speciation and/or competition for binding sites, as was suggested for killifish by Blanchard and Grossell (2005).

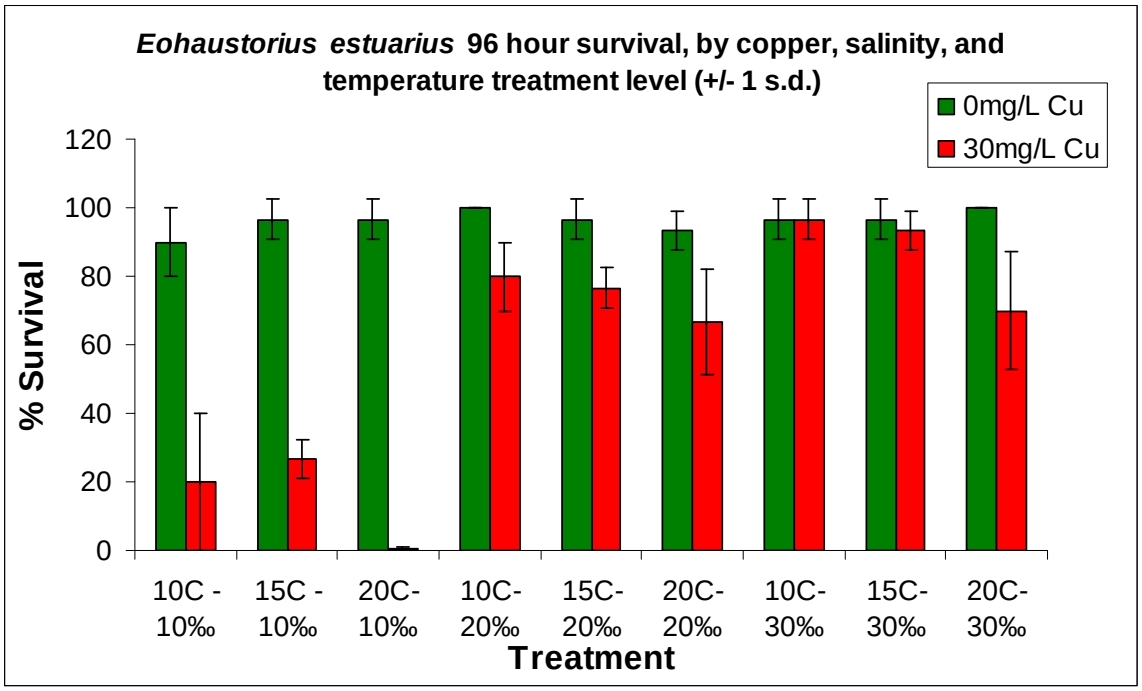

Figure 3-2. Mean percent survival (± 1 s.d.) of the estuarine amphipod *Eohaustorius estuarius* following 96 hour exposures in clean seawater or 30 mg/L nominal Copper under varying salinity and temperature combinations.

Table 3-2. ANOVA results (P-values), *L. plumulosus* and *E. estuarius* survival. Statistically significant (p<0.05) results listed in bold.

Factor	Df	*L. plumulosus*	*E. estuarius*
Copper	1	**<0.0001**	**<0.0001**
Temperature	2	**0.011**	**0.01**
Salinity	2	**<0.0001**	**<0.0001**
Cu:Temp	2	0.12	**0.01**
Cu:Salinity	2	**0.0001**	**<0.0001**
Temp:Salinity	4	**0.002**	0.52
Cu:Temp:Salinity	4	**0.03**	0.18

3.4. *Leptocheirus plumulosus* Copper LC50 Determination

Due to the lack of aqueous Cu toxicity data available for *L. plumulosus* in the peer-reviewed literature, a multi-concentration test was conducted prior to the salinity/temperature experiment. Cu concentrations (from $CuSO_4$ salts) ranged from 62.5 µg/L to 10 mg/L on a nominal basis. Exposure was conducted for 96 h at a salinity of 20 ‰ and temperature of 20 °C, which are close to recommended standard test conditions for this species (USEPA 1994). The LC50 value based on nominal concentrations was 886 µg/L, while the measured (dissolved) LC50 value was 461 µg/L (Figure 3-3). The lower dissolved concentration is not surprising, as Cu was observed to have precipitated out at the higher test concentrations, which has been reported for other copper studies conducted in saltwater (McPherson and Chapman 2000).

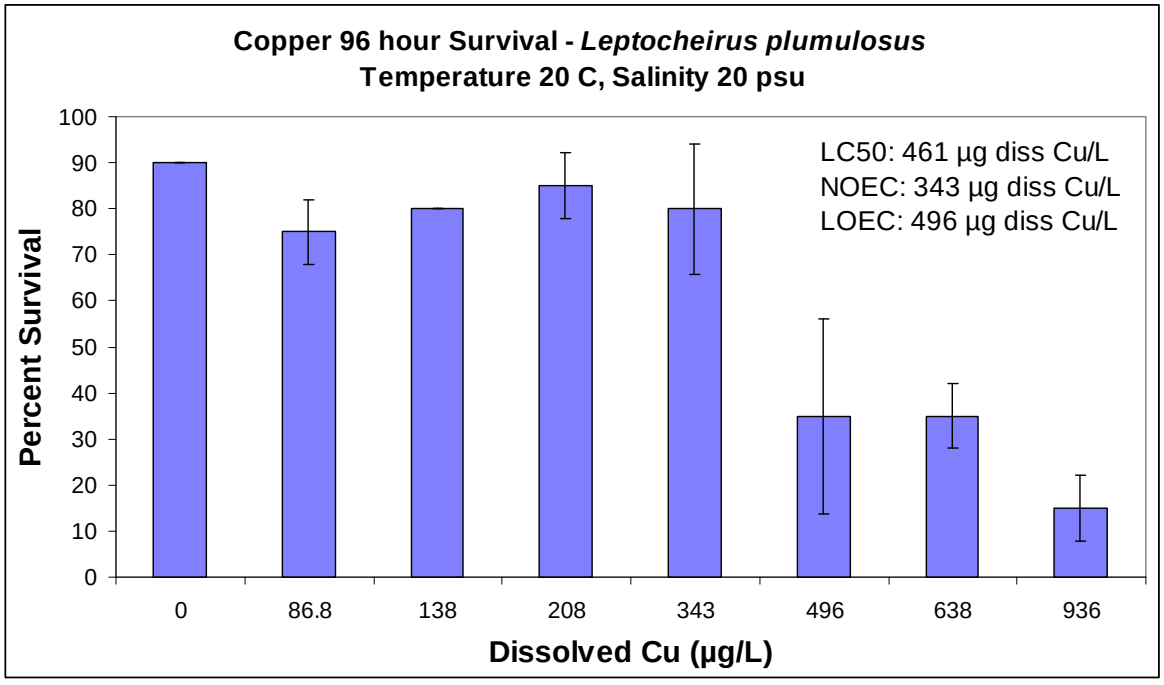

Figure 3-3. Results from copper range-finding experiment for estuarine amphipod *Leptocheirus plumulosus*.

3.5. Amphipod (*L. plumulosus*) Salinity/Temperature Experiment

The subsequent salinity/temperature experiment for *L. plumulosus* was conducted using both uncontaminated seawater and uncontaminated seawater consisting of a single nominal Cu concentration of 800 µg/L, targeting the LC50. Results are shown in Figure 3-4.

Survival was high in the absence of copper for most treatments, but decreased survival was observed at the highest salinity at both 20 and 25 °C. The survival of *L. plumulosus* was most impacted at the warmest temperature and highest salinity evaluated (25 °C, 30 ‰). It is unclear as to why this occurred, as these conditions are reportedly within the range of that tolerated by *L. plumulosus* (ASTM 2000). Animals were cultured, however, at a test temperature of 20 °C and salinity of 20 ‰, where survival was 100%.

In the presence of copper, ANOVA showed significant differences with respect to salinity and an interaction between salinity and temperature, but temperature alone did not explain observed differences. Sensitivity increased with increasing salinity at the lowest test temperature and resulted in unimodal responses with increasing salinity at the remaining temperatures. At 20°C and 25°C, it is interesting to note that magnitude of sensitivity to salinity was greater at low salinities than at high salinities, which was statistically significant at the highest temperature according to the Tukey test. This is similar to the observations made with *E. estuarius*, where lowest survival was observed for all salinities. The unimodal responses with salinity for *L. plumulosus* could be associated with iso-osmotic pressure. Hall et al. (2008) noted that reduced sensitivity of copper was observed at the iso-osmotic salinity for the estuarine copepod *Eurytemora affinis*, with increased sensitivity below and above that salinity.

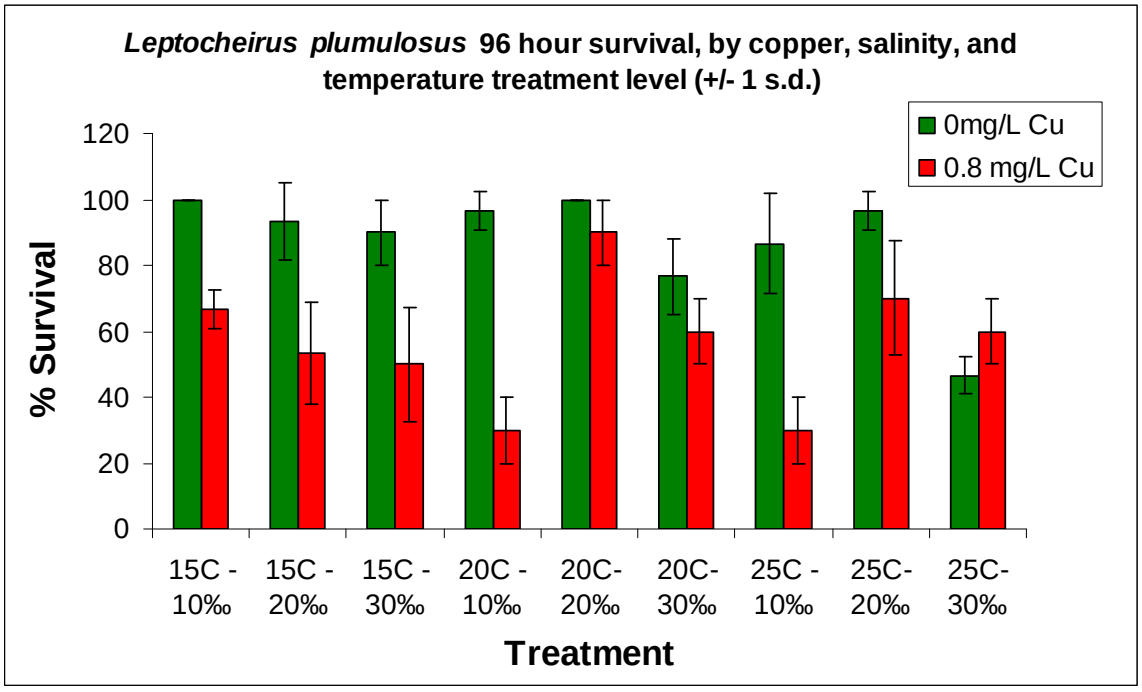

Figure 3-4. Mean percent survival (± 1 s.d.) of the estuarine amphipod *Leptocheirus plumulosus* following 96-hour exposures in clean seawater or 800 µg/L Copper under varying salinity and temperature combinations.

The following tables show all statistical analyses for the *L. plumulosus* experiments:

Table 3-3. Two-way ANOVA models for Cu=0 and Cu=0.8. Survival = Temp + Salinity + Temp:Salinity. (Proportions of survivors were arcsin square root transformed for this and all subsequent analyses). Df=degrees of freedom; p-value < 0.05 indicates statistical difference for the parameter(s).

Factor	Df	SumSq	MeanSq	F-ratio	p-value
Cu=0 µg/L					
Temperature	2	0.361	0.180	5.289	**0.016**
Salinity	2	1.035	0.518	15.174	**0.0001**
Temp*Salinity	4	0.286	0.071	2.096	0.124
Residuals	18	0.614	0.034		
Cu=800 µg/L					
Temperature	2	0.050	0.025	1.143	0.341
Salinity	2	0.524	0.262	12.023	**0.0005**
Temp*Salinity	4	0.626	0.157	7.188	**0.0012**
Residuals	18	0.392	0.022		

Table 3-4. Tukey comparison of means tests for all Temp:Salinity comparisons, by copper treatment level. Non overlapping letters (A,B,C) following each Temp:Salinity combination indicate differences among means at P<0.05.

Temp	Salinity	Mean % Survival	S.D.	Tukey
Cu=0				
15	10	100	0	A
15	20	93.3	11.5	A
15	30	90	10	A
20	10	96.7	5.8	A
20	20	100	0	A
20	30	76.7	11.5	AB
25	10	86.7	15.3	AB
25	20	96.7	5.8	A
25	30	46.7	5.8	B
Cu=800 µg/L				
15	10	66.7	5.8	ABC
15	20	53.3	15.3	AC
15	30	50	17.3	AC
20	10	30	10	A
20	20	90	10	BC
20	30	60	10	ABC
25	10	30	10	A
25	20	70	17.3	BC
25	30	60	10	BC

Table 3-5. Survival data from both copper treatment levels were combined into a single 3-factor ANOVA (Proportions of survivors were arcsin square root transformed prior to these analyses). Df=degrees of freedom; p-value < 0.05 indicates statistical difference for the parameter(s).

Factor	Df	SumSq	MeanSq	F-ratio	p-value
Copper	1	2.79	2.79	99.97	**0**
Temperature	2	0.28	0.14	5.08	**0.011**
Salinity	2	0.90	0.45	16.12	**0**
Cu:Temp	2	0.13	0.06	2.27	0.12
Cu:Salinity	2	0.66	0.33	11.77	**0.0001**
Temp:Salinity	4	0.57	0.14	5.10	**0.002**
Cu:Temp:Salinity	4	0.34	0.09	3.06	**0.03**
Residuals	36	1.01	0.03		

3.6. Mussel embryo-larval development salinity/temperature experiment

Embryo-larval development salinity and temperature experiments with the Mediterranean mussel (*Mytilus galloprovincialis*) incorporated multi-concentration tests with copper, which provided 48 hour EC50 values for each of the combinations, as opposed to data for just one concentration. Because of this test's rather high sensitivity to copper, it would have been difficult to select one exposure concentration that best represented the variation in effects of copper across all treatment combinations. Toxicity testing followed standard protocols (USEPA 1995, ASTM 1999). Nominal copper concentrations were 0, 1.9, 3.8, 7.5, 15, and 30 µg/L, and were based on previous data from our lab for tests conducted at 15 °C and 30‰ in filtered coastal seawater (EC50 ~6.5 µg/L; Rosen et al. 2005).

The top graph in Figure 3-5 indicates that normal larval development success rate was generally very high. Percent normal survival (number normally developed live specimens relative to initial number of embryos added) test acceptability is 70% (ASTM 1999), which was achieved for all combinations, except the 10°C/25‰ combination. The coldest test temperature also mildly impacted development success at 35‰. It should be noted, however, that all the 10 °C treatments required an additional 24 hours (for a total exposure duration of 72 h) to develop to the prodissoconch I stage (D-shaped hinged larval shell) of development, while the standard 48 h exposure was sufficient for the other temperatures. This is particularly important when considering using this test endpoint in field exposures, as was also noted by His et al. (1989).

Copper EC50s varied 3-fold, with the lowest salinity always resulting in the most toxicity (lowest EC50) for any given temperature. Low salinity in the presence of copper has also been shown to reduce normal development of oyster (*Crassostrea virginica*) embryos (MacInnes and Calabrese 1979). As speculated earlier for *E. estuarius*, the lower salinity treatments likely resulted in increased bioavailability of copper. In addition, the lowest salinity tested approaches the limits of tolerance for this species, which may suggest some degree of physiological stress at this salinity. A reduction in sensitivity was also noted with increasing temperature. This may be associated with higher metabolism at higher temperature. The observed more rapid developmental rate to the D-stage at warmer temperatures reduced the time of exposure to copper at the earliest stages of cell differentiation, which are likely the most sensitive.

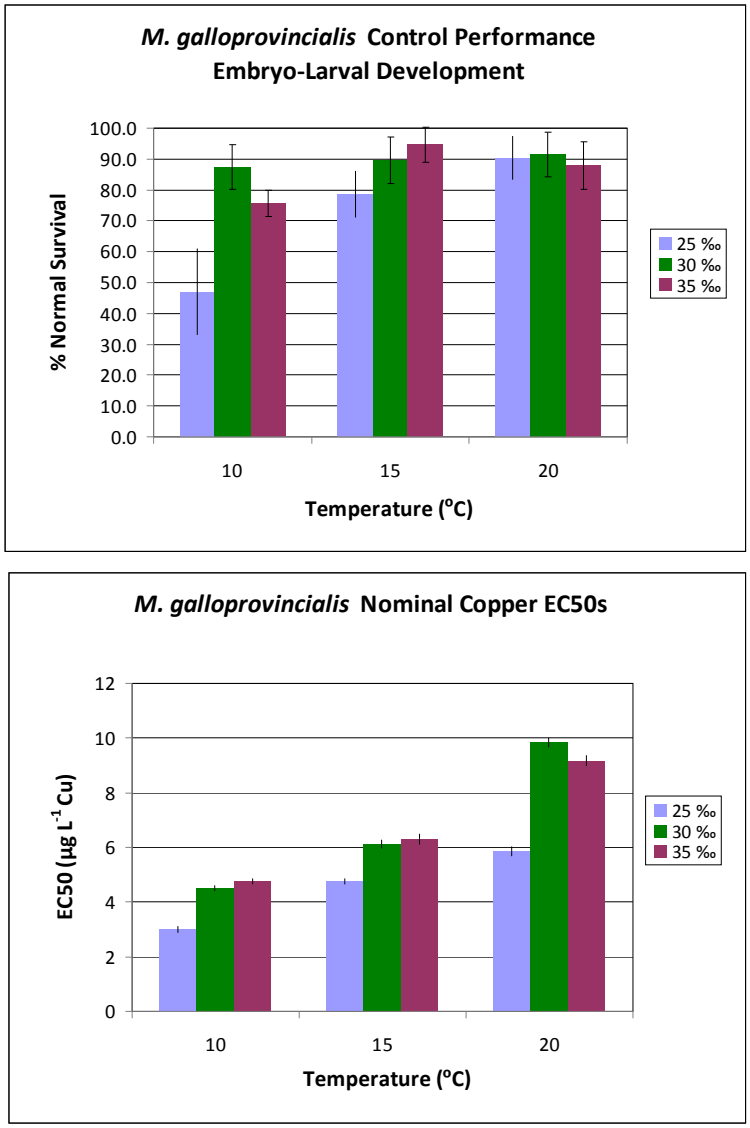

Figure 3-5. Mean control performance (± 1 s.d.)(top figure) and EC50 values (± 95% C.L.)(bottom figure) for mussel (*Mytilus galloprovincialis*) embryo-larval development following 48 h exposures in clean or copper-spiked seawater under varying salinity and temperature combinations.

3.7. Bioluminescence Reduction (QwikLite) Salinity/Temperature Experiment

Effects of varying salinity and temperature in the presence or absence of copper was also evaluated with the QwikLite bioluminescence test. All testing utilized the cosmopolitan bioluminescent dinoflagellate *Pyrocystis lunula*. This species is currently the species of choice with the QwikLite 200 test unit (Assure Controls 2007). The guidance suggests an optimal temperature range of 17-27 °C, and optimal salinity range of 30‰-35‰. Uncontaminated synthetic sea water (Crystal Sea MarineMix) and synthetic seawater spiked to a nominal Cu concentration of 125 µg/L, based on recently conducted multi-concentration tests were used.

The endpoint for the QwikLite test is a reduction in bioluminescence (light) output from cells exposed for 24 h to a toxicant. Light output is expressed as the total light emitted from the dinoflagellates over a given excitation period (bioluminescent dinoflagellates emit light upon mechanical stimulation), and is

frequently converted into a percentage relative to light output produced from cells exposed in a clean seawater control. Alternatively, data are expressed as the total mean bioluminescence recorded by the photodiode after a specified period of agitation.

In the absence of copper, bioluminescence output was highest under conditions closest to those typically used in QwikLite tests, in this case 22°C/35‰. The dinoflagellates (*Pyrocystis lunula*) also appeared to be unaffected at a temperature of 30 °C at the high salinity range, but output was somewhat inhibited at 15 °C. Reduced bioluminescence for *P. lunula* at lower temperatures was also reported by Craig et al. (2003), where re-establishment of bioluminescence was used as the test endpoint.

For all three temperatures tested in this study, a trend of reduced light output with decreasing salinity was apparent, with the lowest values under the 30°C/15‰ combination (Table 3-6, Figure 3-6). This is not surprising, considering *P. lunula* is an oceanic species, and reductions in bioluminescence have been observed for this species in the laboratory at low salinity by others (Craig et al. 2003). The 125 µg/L Cu additions, based on previous testing with this species (Heimann et al. 2002), resulted in significant light output reduction under most combinations, with the largest reductions relative to the respective controls generally observed at the lowest temperatures, and the smallest differences at the higher temperatures. This difference is not as marked as those observed for some other organisms (e.g. *E .estuarius*), and generally contrasts with observations with other species reported herein as well. *P. lunula* is typically found in temperate to sub-tropical areas, and therefore, physiological stress could play a role in reduced light output at 15 °C, as well as an increased response to copper addition at the lowest temperature.

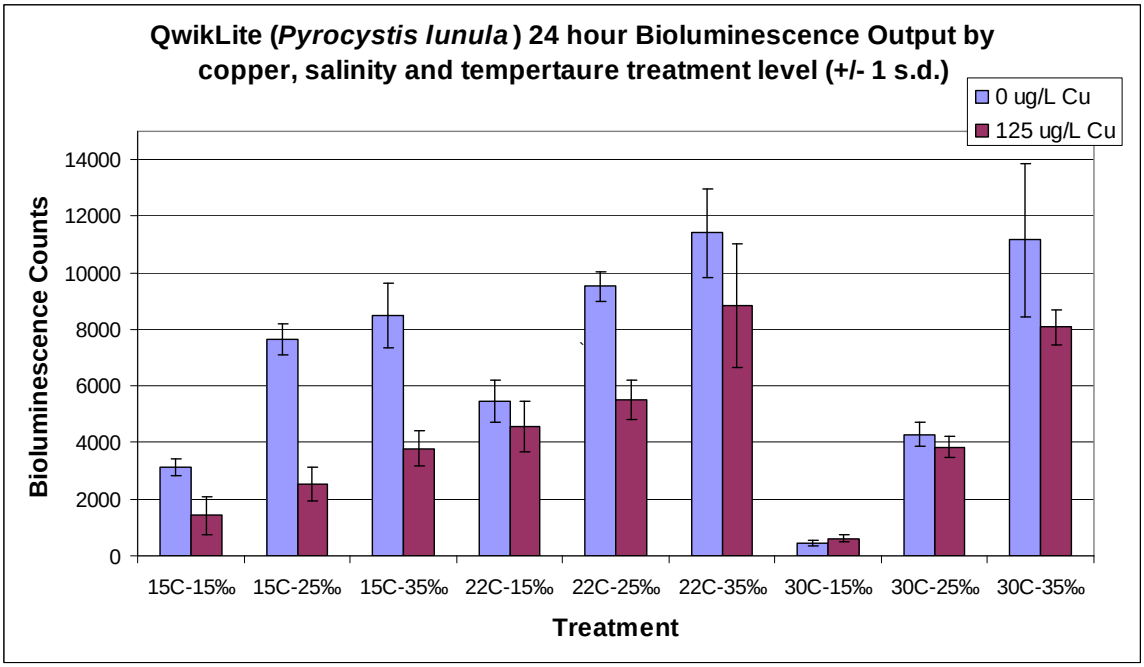

Figure 3-6. Mean bioluminescence (± 1 s.d.) output for the marine dinoflagellate *Pyrocystis lunula* following 24 hour exposures in clean seawater or 125 µg/L nominal copper under varying salinity and temperature combinations.

Table 3-6. ANOVA Results, Qwiklite Cu Screening Tests.

	Df	Sum Sq.	Mean Sq.	F ratio	P(F)
Three-Way ANOVA, Qwiklite Cu Screening Test (Effects of Temperature, Salinity, and Copper Treatments) on Bioluminescence					
Temperature	2	207129245	103564622	92.4	0
Salinity	2	655482916	327736458	292.4	0
Copper	1	166306411	166306411	148.4	0
Temp:Salinity	4	103662722	25915681	23.1	0
Temp:Copper	2	33039092	16519546	14.7	0
Salinity:Copper	2	38265617	19132809	17.1	0
Temp:Salinity:Cu	4	15333127	3833282	3.42	0.01
Residuals	90	100885525	1120950		
Two-Way ANOVA, Qwiklite Screening Test (Effects of Temp and Cu at 15°C)					
Salinity	2	96518124	48259062	101.0	0
Copper	1	132583710	132583710	277.6	0
Salinity:Copper	2	21186381	10593191	22.2	0
Residuals	30	14329473	477649		
Two-Way ANOVA, Qwiklite Screening Test (Effects of Temp and Cu at 22°C)					
Salinity	2	157519252	78759626	51.0	0
Copper	1	55299053	55299053	35.8	0
Salinity:Copper	2	14541416	7270708	4.7	0.02
Residuals	30	46332479	1544416		
Two-Way ANOVA, Qwiklite Screening Test (Effects of Temp and Cu at 30°C)					
Salinity	2	505098262	252549131	188.4	0
Copper	1	11462739	11462739	8.5	0.007
Salinity:Copper	2	17870947	8935474	6.7	0.004
Residuals	30	40223573	1340786		

Notes: Optimal temperature range is 17°C-27°C.
Optimal salinity range is 30‰-35‰
Test was conducted on 3 consecutive days. On each of the 3 days, all Cu and salinity combinations were tested at one temperature.

3.8. Polychaete Post Exposure Feeding Copper EC50 Determination

Polychaete post exposure feeding rate was deemed a good candidate as a relatively rapid sublethal toxicity test for exposure to bulk sediment in the literature review, primarily because of its ecological relevance to evaluations of surficial sediment, short exposure period, and apparent sensitivity based on published data using a similar species *Hediste diversicolor* (Moreira et al. 2005). Initial experimentation using *Neanthes arenaceodentata,* which is more commonly found and tested in North America were based on the Moreira et al (2005) approach. Because of their small size at test initiation in the standard 20-28 day survival and growth tests, two *N. arenaceodentata* sizes classes were considered for initial testing: 2-3 week emergent juveniles (small), and 6-8 week old adults (large). Effects of copper on feeding rate involved 96 h exposures in aqueous solutions, followed by a 1-2 hour (depending on size) feeding period on *Artemia* (brine shrimp) nauplii in clean seawater. Copper was added to uncontaminated filtered seawater at 7 different concentrations ranging from 7 to 150 µg/L, based on a previously reported 96 h LC50 value of 77 µg/L (Reish and Gerlinger 1997).

Experiments were conducted under static renewal conditions in 400 ml beakers consisting of 250 ml of test solution. Five worms were added to each of 3 replicate beakers for each concentration. Testing was conducted at a temperature of 20 °C and a salinity of 30 ‰. Upon final examination of survivors at 96 h,

6 worms from each treatment (two from each beaker) were then allowed to feed for the designated time. Feeding rate was expressed as mean *Artemia* consumed.

Survival, LC50 values and post-exposure feeding rate EC50 values are shown in Figure 3-7 and Table 3-7. LC50 and EC50 values were lower for the small (2-3 week old) worms, suggesting higher sensitivity. Relatively large confidence intervals around the point estimates, however, were noted. A reduction in variability is currently being addressed with this assay by making various modifications to the developing protocol. Although the small worms were apparently more sensitive to cooper in this assay, large (6-8 week old) worms showed greater sensitivity in the pore water comparative study. In addition, large worms appear to more reliably consume the food within a reasonable time period. Therefore, larger worm were utilized in subsequent portions of this project. Large worms are also ideal because the increased tissue mass is useful for tissue analyses.

Table 3-7. Median lethal concentrations (LC50) and median effects concentrations (EC50) based on post-exposure feeding of the polychaete *Neanthes arenaceodentata* from exposures to copper in aqueous solutions.

	Metric (µg/L)		
Worm age (weeks)	**48 h LC50**	**96 h LC50**	**Feeding EC50**
2-3	67.3 (52.4-86.5)	56.7 (42.3-75.9)	25.2 (14.0-42.4)
6-8	118.7 (101.2-139.3)	79.9 (61.5-104)	76.0 (38.6-150)

Nominal copper concentrations. Measured concentrations were determined in subsequent experiments using this assay (Section 7).

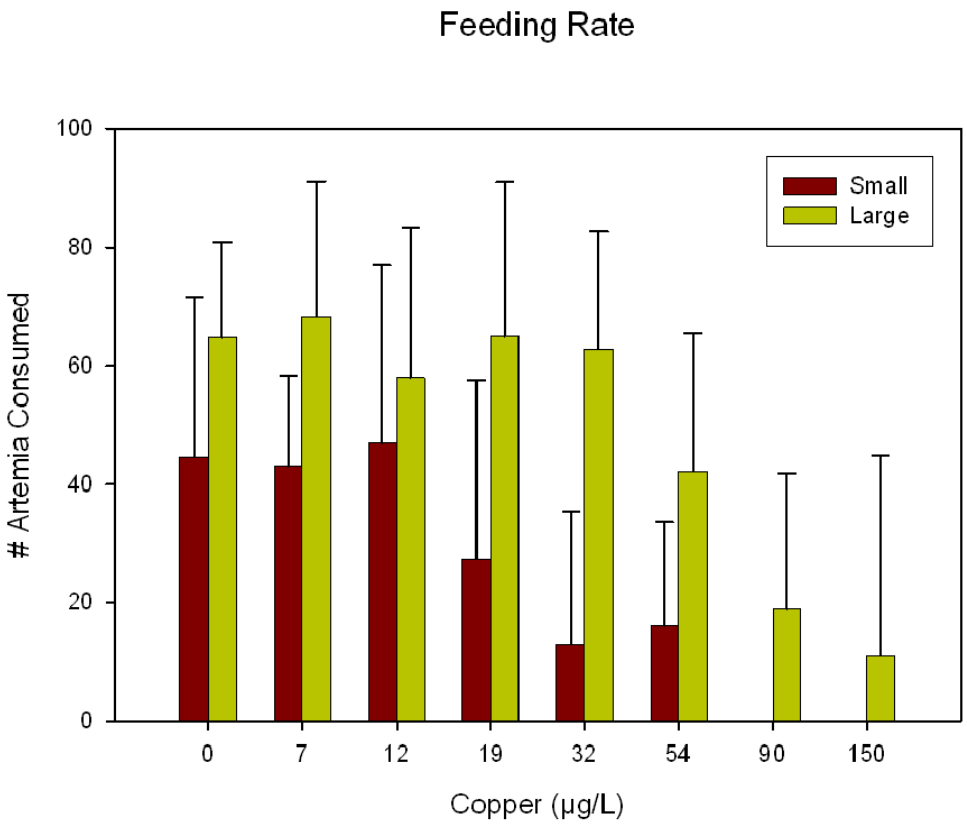

Figure 3-7. Response of small (2-3 week old) and large (6-8 week old) polychaetes (*Neanthes arenaceodentata*) in developmental post-exposure feeding rate assay following 96 hour exposure to copper.

3.9. Conclusions

As was expected, responses to different salinity and temperature combinations on the toxicity of copper differed among the different test endpoints. In general, however, toxicity was greatest at the lowest test salinities, which might be linked to increased uptake of copper as a consequence of speciation and/or competition for binding sites. Increasing temperature increased toxicity to *E. estuarius*, which could be due to fairly dramatic impacts on the solubility of copper at the relatively high concentrations that were exposed to this species. Organism metabolism also appeared to play a role in the observance of effects, with the more rapid development of mussel larvae at higher temperatures reducing toxicity, which would have reduced exposure time to developing embryos during the most critical stages of cell differentiation. Physiological stress may also have played a role in some cases. For *L. plumulosus*, reduced copper toxicity at the mid-range salinity may have been associated with the iso-osmotic salinity for this species (or at least salinity under which the organisms are cultured), which has been observed for other estuarine crustaceans. Intolerance to certain salinities and temperatures, and the effects of salinity and temperature on metal bioavailability should be taken into account when developing an approach to *in situ* testing.

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4.0 POREWATER SCREENING EVALUATION

The proposed SEAP approach is allow for the option of broad-scale pore water sampling using the Trident, with laboratory-based pore water bioassays being conducted. The results from these bioassays can assist in the selection of sites for more intensive in situ evaluations using the SEA Ring. In November and December of 2007, the short list of potential laboratory and *in situ* screening assays were evaluated for relative sensitivity and performance according to previously identified evaluation criteria (Section 2.0). The approach to this evaluation occurred in two phases, with a smaller scale experiment designed to confirm that successful toxicity testing could be conducted on *in situ* collected pore water samples, and a larger scale experiment designed to concurrently evaluate all selected tests in a range of samples and expected degrees of contamination.

4.1. First Experiment (Small Scale)

The first experiment was conducted in November 2007, with the purpose of troubleshooting any problems that might occur in using the Trident Probe to collect sufficient quantities of pore water required to conduct the multiple toxicity test methods that would be required in the full scale study, as well as enough water to conduct relevant chemical analyses without sacrificing method detection limits. It was also critical to confirm the absence of any artifacts that might influence toxicity that might be associated with components or sampling processes associated with the Trident.

Sampling occurred at a reference site (SB2441) near the mouth of San Diego Bay. This site has been used in risk assessments previously, showing background contaminant concentrations and no significant toxicity to amphipods or mussel embryos in standard laboratory solid phase and sediment water interface tests, respectively. The site was also ideal because it allowed for the collection of relatively uncontaminated pore water from a gradient of sediment grain sizes, as our sampling vessel moved away from the adjacent sandy beach. It was deemed that use of sand packs over the standard probe greatly increased the ability to remove pore water. Also improving the ability to collect relatively large sample volumes was increasing the number of sampling probes on the Trident to a total of three (Figure 4-1). A total of 500 mL of pore water was targeted at each site for the small scale testing effort. This was achieved without too much difficulty in a range of grain sizes.

Figure 4-1. Photos of Trident Probe during November sampling event, illustrating use of multiple pore water sampling probes and sand packs to increase flow rate in finer grained sediments.

Toxicity testing was conducted on a subset of the available screening test methods, and included the dinoflagellate (*Pyrocystis lunula*) bioluminescence test (QwikLite), sea urchin (*Strongylocentrotus purpuratus*) fertilization, and the developmental polychaete (*Neanthes arenaceodentata*) feeding rate assay. There was no particular reason that these three tests were collected, other than that they are all small volume tests, and it was uncertain as to how much sample could be obtained from the Trident pore water samplers. All samples were within range of normal salinity, temperature, and dissolved oxygen (DO) requirements during toxicity testing, but some samples were characterized as having below acceptable DO concentrations upon collection. Sample PW3, which was more fine-grained in nature than the other two samples, had a relatively high initial total ammonia concentration (Table 4-1). The total ammonia concentration dropped from 12.6 to 8.5 mg/L upon toxicity test setup, however, and was not expected to be an issue for any of the tests, based on their thresholds for ammonia (Rosen et al. 2009), and the lack of any noticeable problems with pH that would have resulted in elevated unionized ammonia concentrations.

In general, toxicity was not observed in the field samples, nor from a variety of equipment blank preparations (Figure 4-2 through Figure 4-4). The apparent toxicity in sample PW3 to sea urchin fertilization success could not be explained by the relatively high ammonia concentration measured in that sample. The overall results illustrated success in the ability to collect sufficient pore water volume *in situ* using this method, in adedition to successful laboratory testing of the samples with no apparent artifactual issues.

Table 4-1. Water quality measurements on pore water samples made in the field (pH, salinity, temperature, dissolved oxygen [D.O.]) following collection using the Trident Probe or upon arrival in the laboratory.

Sample ID	Nominal Sediment Type	pH	Salinity (psu)	Temp. (deg C)	D.O. (mg/L)	Total Ammonia (mg/L)
PW1	Sandy	7.28	33.3	20.9	4.84	0.122
PW2	Medium	7.43	32.5	19.1	3.44	3.3
PW3	Fine	7.78	32.4	21.5	4.02	12.57

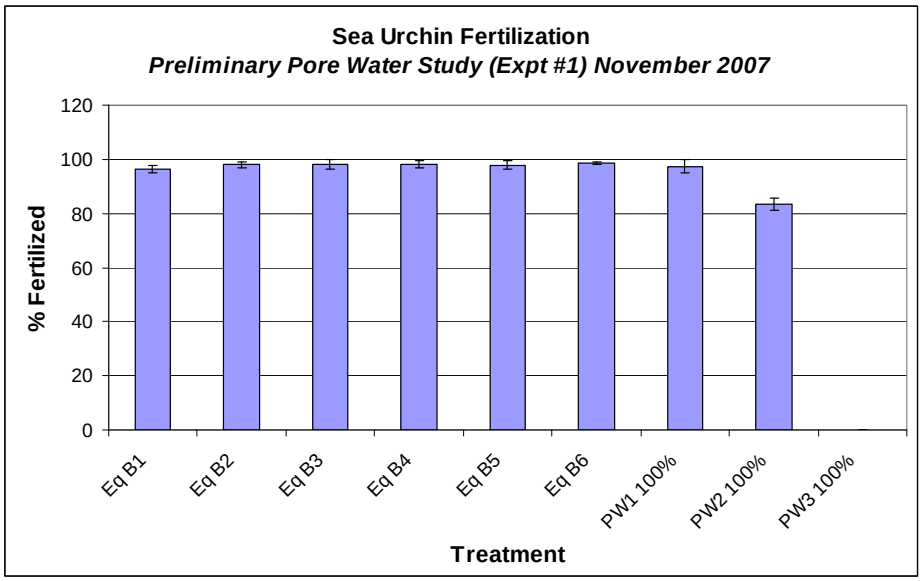

Figure 4-2. Sea urchin (*Strongylocentrotus purpuratus*) fertilization test results from preliminary pore water study at reference site SB2241. Eq B samples are Trident Probe equipment blanks rinsed with clean natural seawater. PW1, PW2, and PW3 are pore water samples collected using the Trident.

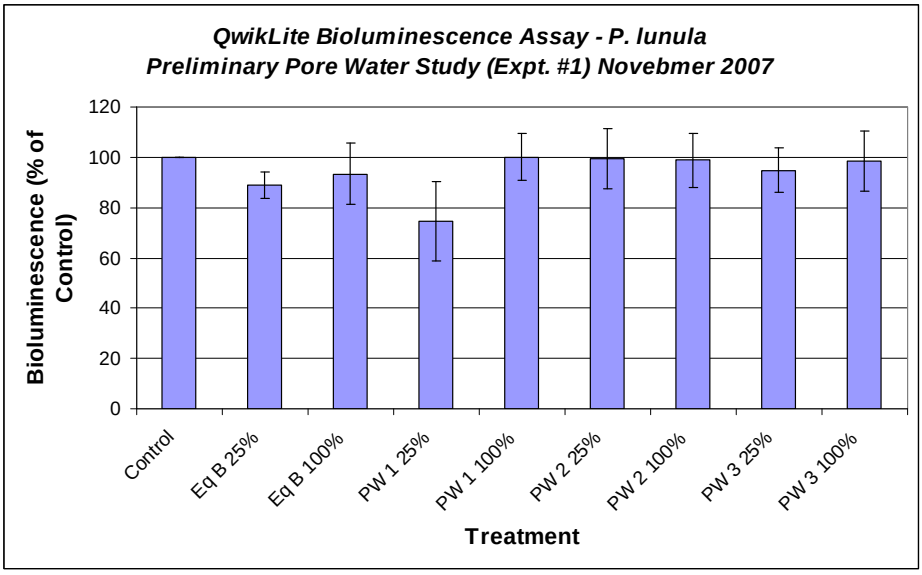

Figure 4-3. QwikLite (*Pyrocystis lunula*) test results from preliminary pore water study at reference site SB2241. Eq B sample is Trident Probe equipment blank rinsed with clean natural seawater. PW1, PW2, and PW3 are pore water samples collected using the Trident.

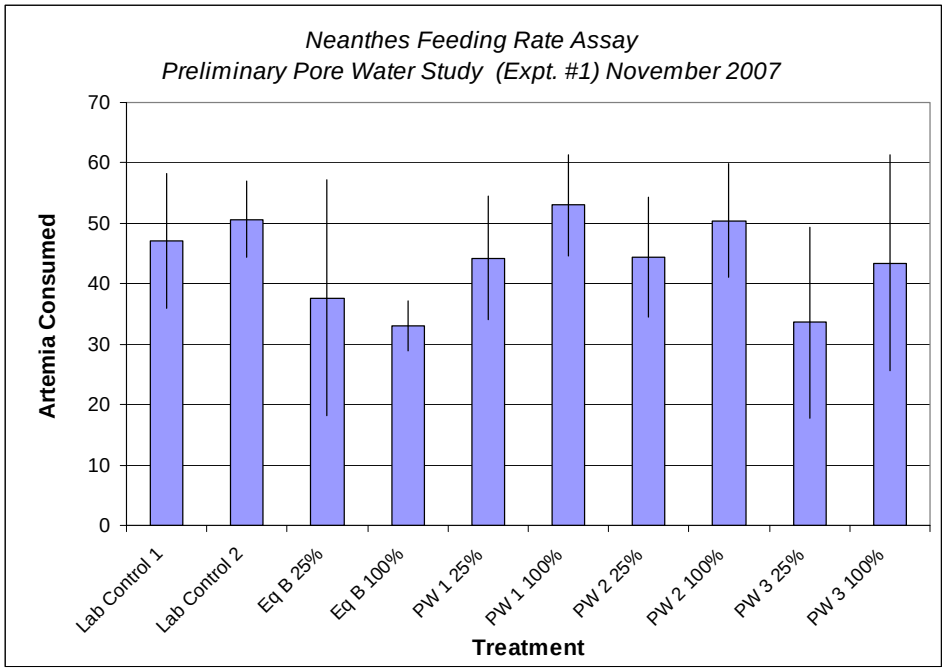

Figure 4-4. Developmental polychaete (*Neanthes arenaceodentata*) post exposure feeding rate test results from preliminary pore water study at reference site SB2241. Eq B sample is Trident Probe equipment blank rinsed with clean natural seawater. PW1, PW2, and PW3 are pore water samples collected using the Trident.

4.2. Second Experiment (Full Scale)

The second pore water screening experiment included the full suite of 'short list' *in situ* toxicity tests on a larger number of samples which ranged in expected degree of contamination. A total of seven samples were collected *in situ* from San Diego Bay using the Trident Probe. Three of these samples were collected at Naval Station (NAVSTA) San Diego (NS11 F, NS14 F, NS24), a Navy site that has previously been characterized as being moderately contaminated (PAHs, PCBs, pesticides, and metals) and as moderately toxic (Chadwick et al. 1999), one at Shelter Island (SI21), a marina in the San Diego Bay that is currently on the CWA Section 303(D) list for impairment by copper, and three sites (SB2441, SB90056, CP2243) previously characterized as relatively uncontaminated and historically used as reference sites in ecological risk assessments. Two of these samples were spiked with copper targeted at concentrations relevant to thresholds for the individual test types, and two additional bulk sediment samples from NAVSTA were collected and centrifuged in the laboratory. This resulted in a total of 11 pore water samples. The Sample locations and physico-chemical parameters measured upon collection are provided in Table 4-2.

Table 4-2. Field details for Trident pore water samples collected for full-scale screening bioassay experiment.

FIELD ID	SI21	SB2441	SB90056	CP2243	NS11	NS14	NS24
Sample Collect Date	4-Dec-07	4-Dec-07	4-Dec-07	5-Dec-07	5-Dec-07	6-Dec-07	6-Dec-07
Sample Collect Time	1021	1255	1449	0958	1720	1320	1622
Sample Anal. Time (wq)	1023	1257	1451	0959	1722	1322	1624
WAAS Diff GPS Lat	32° 43.607	32° 41.507	32° 43.157	32° 39.879	32° 40.895	32° 40.811	32° 40.625
WAAS Diff GPS Long	117° 14.011	117° 14.327	117° 13.046	117° 8.563	117° 7.621	117° 7.827	117° 7.606
WAAS Diff GPS Acc (ft)	6.9	6.9	13.0	7.0	9.2	8.0	15.0
Water Depth (ft)	4.2	19.0	23.9	13.9	28.4	38.5	32.1
Trident							
Penetration Depth (in)	16 bgs	16 bgs	16 bgs	16 bgs	16 bgs	16 bgs	16 bgs
Sediment Depth Profile	sandy	silty sand	sandy	sandy silt	fine mud	fine silt	silty fine
Configuration	3 probe	3 probe	3 probe	3 probe	3 probe	3 probe	3 probe
	No sand	No sand	No sand	No sand	Sand	Sand	Sand
Water Quality							
Conductivity (mS/cm)	44.00	49.14	49.49	49.94	50.05	49.58	51.02
Temperature (°C)	19.7	19.4	17.7	15.6	15.4	18.8	16.3
Salinity (ppt)	28.00	31.67	32.00	32.41	32.49	32.03	32.44
ORP (mV)	103	96	-51	-33	60	45	145
pH	7.33	7.58	7.47	7.47	7.57	7.34	8.12
DO (mg/L)	3.81	2.08	2.26	2.76	na	na	6.92

Pore water samples were evaluated for toxicity against a total of 10 toxicity test types (9 test species). Testing commenced within 1 week of sample collection. Samples were held on ice in the field, and stored in the dark at 4 °C until testing. Exposures of all screening tools were conducted concurrently. When applicable, standard test methods were used. Modifications to some methods, however, were required. All tests were conducted in sample volumes of 10 ml per replicate or less (

Table 4-3). This volume is consistent with sea urchin fertilization, mussel embryo, rotifer, and QwikLite standard tests methods, but required a reduction in the typical chamber size and test volume for mysids and amphipods. Amphipods and mysids, however, have been tested successfully in small volumes, particularly for toxicity identification evaluations (TIE) using pore waters (USEPA 1996, Ho et al. 1997, Anderson et al. 2007). At the time of this study, the developmental polychaete postexposure feeding assay had not yet been standardized to any specified test volume. Exposure times followed standard guidelines for all tests. All samples were tested at ambient salinity (~32 ‰ for most samples), which is acceptable (within range tolerated) for all test methods employed. Pore water was tested undiluted (100%) and at a 25% dilution. All methods also included a negative control consisting of the dilution water (uncontaminated filtered, 0.45 µm, seawater). Data were compared to controls for statistical differences using t-tests. A sample was considered toxic if it was statistically different from the control and was also less than 80% of the control. The latter criterion was loosely based on the minimum significant difference (MSD) threshold concept that is often used in sediment toxicity evaluations in the risk assessment process (Thursby et al. 1997, Phillips et al. 2001).

4.2.1. Control Performance

With one exception, all toxicity tests were deemed successful, based on performance in the negative controls (uncontaminated 0.45 µm filtered seawater) (Figure 4-5). Most test endpoints ranged from 80 to close to 100% in the controls. Water quality (pH, temperature, dissolved oxygen, and salinity) remained within acceptable levels for all tests. *L. plumulosus* survival was quite variable upon examination after 10

days of exposure, however, so only data for 2 and 4 day exposures for this species were deemed acceptable. The mortality at 10 days could be due to starvation, as amphipods were not fed. Previous pore water testing with *E. estuarius*, as part of toxicity identification evaluations (TIEs), have not included feeding (Anderson et al. 2007), but no 10-day exposures in pore water only (no sediment) were found in the literature for *L. plumulosus*. The overall conclusion from control performance, however, is that all of the tested species can be successfully exposed in small volumes (Figure 4-6). It is imperative, however, that water quality be closely monitored, and that considerations with respect to feeding requirements be made.

Table 4-3. List of test method and relevant identifying factors used in the full scale pore water screening experiment.

Test Method & Endpoint	Species	Age or size	Symbol	Exposure (d)
QwikLite bioluminescence	*Pyrocystis lunula*	2 weeks	Q- P.l.	1
QwikLite bioluminescence	*Ceratocorys horrida*	2 weeks	Q-C.h.	1
Sea urchin fertilization	*Strongylocentrotus purpuratus*	< 1 h	S.p.	0.04
Mussel larval development	*Mytilus galloprovincialis*	< 4 h	M.g.	2
Mysid shrimp survival	*Americamysis bahia*	3 day	A.b.	2, 4, 7
Amphipod survival	*Eohaustorius estuarius*	3-5 mm	E.e.	2, 4, 10
Amphipod survival	*Leptocheirus plumulosus*	3-5 mm	L.p.	2, 4, 10
Polychaete feeding rate	*Neanthes arenaceodentata*	6-8 week (lg.)	N.a. Lg	
Polychaete feeding rate	*Neanthes arenaceodentata*	2-3 week (sm.)	N.a. Sm	
Rotifer survival	*Brachionus plicatilis*	< 24 h	B.p.	1, 2

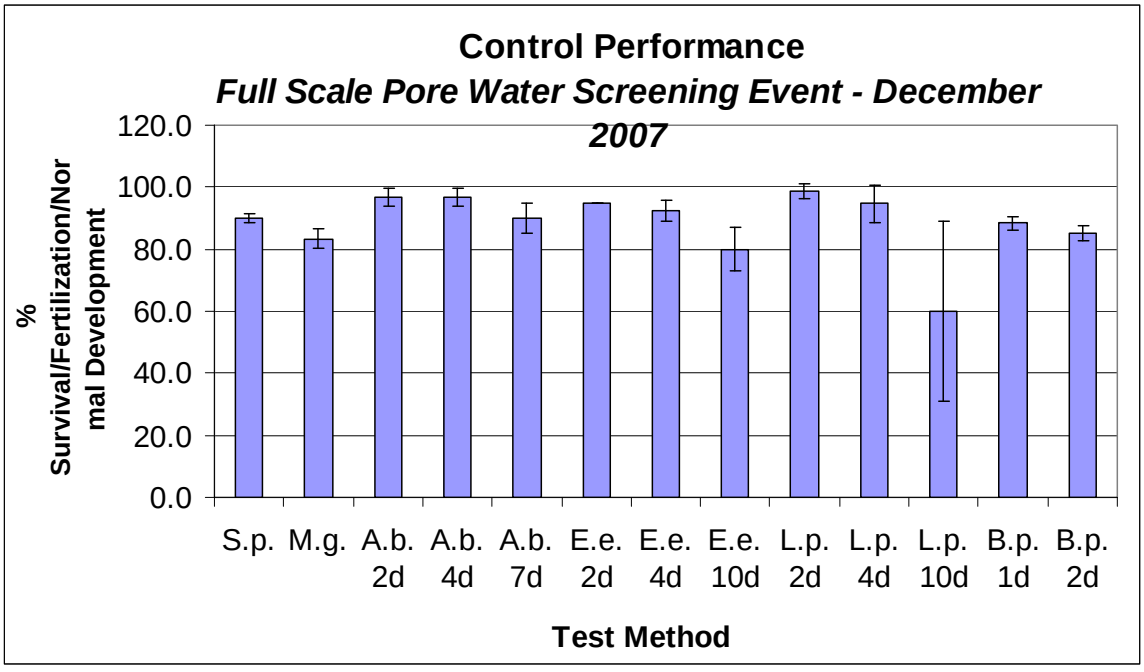

Figure 4-5. Control performance for the different screening tests employed in the full scale pore water screening assessment, ranging in duration fo 1 h through 10 days (d). S.p.=*Strongylocentrotus purpuratus*; M.g.=*Mytilus galloprovincialis*; A.b.=*Americamysis bahia*; E.e.=*Eohaustorius estuarius*; L.p.=*Leptocheirus plumulosus*; B.p.=*Brachionus plicatilis*.

Sample Type	Sample ID	Test Species									% Sig. &
		Q- *P.l.*	Q- *C.h.*	*S.p.*	*M.g.*	*A.b.* 7d	*E.e.* 10d	*L.p.* 4d	*N.a.* Lg	*B.p.* 2d	**< 80% control**
Reference	CP2243										**0**
Reference	SB2441									●	**11**
Reference	SB90056	●	●								**22**
Test	NS11 F	●	◘	●	◘	●					**56**
Test	NS14 F		◘	●	◘						**33**
Test	NS11 C	●	◘		●	●			●		**56**
Test	NS14 C	-	-		●					●	**29**
Test	NS24	●									**11**
Test	SI21	●	●	●	●						**44**
Spiked	CP2243 + Cu	●	●	●		●			●	●	**67**
Spiked	SB2441 + Cu	●	●	●	●	●		●	●	●	**89**
% Samples Toxic		**70**	**70**	**45**	**55**	**36**	**0**	**9**	**27**	**36**	

● Significantly different and < 80% of control

◘ Significantly different and < 80% of control, but likely impacted by ammonia

\- Not enough sample to test

Figure 4-6. Summary of in situ pore water toxicity test results for 9 different test methods. S.p.=*Strongylocentrotus purpuratus*; M.g.=*Mytilus galloprovincialis*; A.b.=*Americamysis bahia*; E.e.=*Eohaustorius estuarius*; L.p.=*Leptocheirus plumulosus*; B.p.=*Brachionus plicatilis*. Values shown at right of Table indicate the percentage of test methods that resulted in toxicity for a given sample, basd on statistically significant differences using equal variance t-tests (significance level=0.05) and values of less than 80% of the appropriate control. The values underneath the table represent the percent of samples that were deemed toxic for a given species basd on the same criteria (t-tests, <80% of control). While dashes indicate there wasn't enough sample to test, empty cells indicate that there were no significan differences.

4.2.2. Pore water toxicity

Overall, pore water samples resulted in moderate to low toxicity. Figure 4-7 shows the mean response for the different tests for all samples tested. Although not necessarily statistically significant, both QwikLite tests ranked relatively high in terms of sensitivity. *C. horrida* was the most sensitive test, but it also was more susceptible to ammonia toxicity (Table 4-4**Error! Reference source not found.**, Table 4-5, Figure 4-6 and Figure 4-7). *P. lunula* is not nearly as sensitive to ammonia (Table 4-5), and removed this potential confounding factor from all samples tested with that species. *P. lunula* was approximately equally as sensitive as standard test methods such as sea urchin fertilization and mussel embryo larval development. It should be noted, however, that conducting the QwikLite test with *P. lunula* is much more cost effective and simpler than the other two tests. Mysids were generally unimpacted in all samples after 2 days of exposure, but sensitivity increased over time, resulting in an average sensitivity ranking after 7 days similar to that for the much shorter QwikLite (*P. lunula*), sea urchin fertilization, and mussel embryo-larval development tests. Neither species of amphipod was particularly negatively impacted by the samples tested. The feeding rate assay was overall less sensitive than most other tests, but responded similarly in terms of relative sensitivity (Figure 4-6, Figure 4-7, Table 4-4 through Table 4-6) to the other species. The rotifer, while comparably sensitive overall (after 2 days of exposure), did not agree with responses for the other test organisms. For instance, the rotifer generally found the reference sites to be somewhat toxic, while NS11 F (the most toxic sample for all other tests; Table 4-4 through Table 4-6), did not affect rotifer survival at all.

Table 4-4. Unionized ammonia thresholds for relevant test organisms used in pore water toxicity tests. Q-P.l.= Qwiklite (*Pyrocystis lunula*); Q-C.h.=QwikLite (*Ceratocorys horrida*); S.p.=*Strongylocentrotus purpuratus*; M.g.=*Mytilus galloprovincialis*; A.b.=*Americamysis bahia*; E.e.=*Eohaustorius estuarius*; L.p.=*Leptocheirus plumulos*us; B.p.=*Brachionus plicatilis*. Empty cells indicate data could not be found in the literature.

Species	Unionized Ammonia (mg/L)			Reference
	NOEC	LOEC	EC50	
S.p.			1.15	Bay et al. 2003
M.g.		0.152	0.12	Phillips et al. 2005
A.b.			0.83	Kohn et al. 1994
E.e.	0.8		2.49	Kohn et al. 1994
L.p.	0.8			USEPA 1994
B.p.			3.1	Ostrensky & Wasielesky 1992
P.l.	0.359	0.718	0.706	unpublished data
C.h.	0.036	0.072	0.192	unpublished data

Table 4-5. Unionized ammonia concentrations measured during screening tests in pore water samples, based on water quality characteristics for various test types. Q-P.l.= Qwiklite (*Pyrocystis lunula*); Q-C.h.=QwikLite (*Ceratocorys horrida*); S.p.=*Strongylocentrotus purpuratus*; M.g.=*Mytilus galloprovincialis*; A.b.=*Americamysis bahia*; E.e.=*Eohaustorius estuarius*; L.p.=*Leptocheirus plumulosus*; B.p.=*Brachionus plicatilis.*

Sample ID	Unionized Ammonia (mg/L)		
	M.g. S.p. E.e.	*L.p. N.a. Q-P.l. Q-C.h.*	*B.p.*
NS11 F	0.422	0.560	0.847
NS14 F	0.188	0.251	0.383
NS11 C	0.090	0.120	0.186
NS24	0.011	0.015	0.023
SI21	0.006	0.007	0.012
CP2243	0.080	0.108	0.166
SB2441	0.010	0.014	0.021
SB90056	0.027	0.037	0.057
CP2243 + Cu	0.092	0.123	0.189
SB2441 + Cu	ND	ND	ND
NS14 C	0.030	0.040	0.063

Table 4-6. Ranking of individual field collected pore water samples in order of decreasing sensitivity to 8 different toxicity test endpoints (1= most toxic, 9= least toxic). Q-P.l.= Qwiklite (*Pyrocystis lunula*); Q-C.h.=QwikLite (*Ceratocorys horrida*); S.p.=*Strongylocentrotus purpuratus*; M.g.=*Mytilus galloprovincialis*; A.b.=*Americamysis bahia*; E.e.=*Eohaustorius estuarius*; B.p.=*Brachionus plicatilis.*

	Test Method							
Sample ID	Q-P.l.	Q-C.h.	S.p.	M.g.	A.b. 7d	E.e. 10d	N.a. Lg	B.p. 2d
CP2243	8	5	6	9	3	7	2	5
SB2441	7	3	7	5	7	8	9	3
SB90056	3	4	4	8	5	9	6	2
NS11 F	1	1	1	1	1	1	1	6
NS14 F	6	2	2	2	4	4	7	8
NS11 C	5	7	8	3	2	5	5	7
NS14 C	-	-	9	6	8	6	3	9
NS24	4	8	5	7	9	3	8	1
SI21	2	6	3	4	6	2	4	4

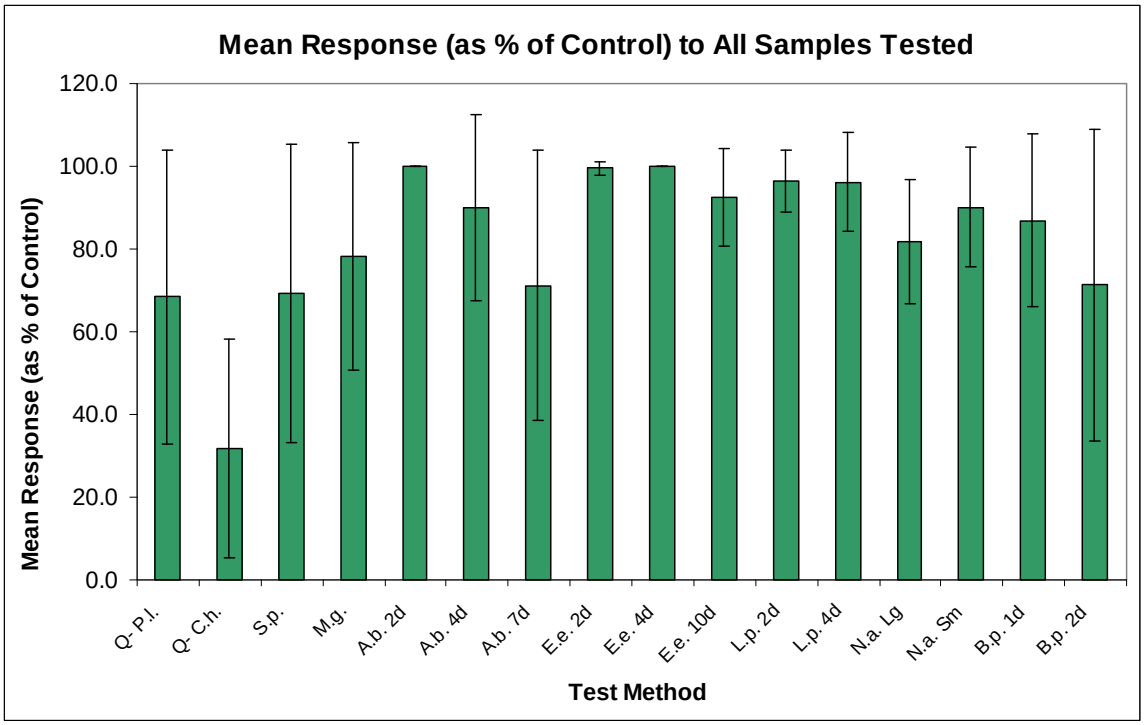

Figure 4-7. Relative sensitivity of each of the test methods to all pore water samples evaluated, expresses as mean response relative to control (± 1 s.d.). Q-P.l.= Qwiklite (*Pyrocystis lunula*); Q-C.h.=QwikLite (*Ceratocorys horrida*); S.p.=*Strongylocentrotus purpuratus*; M.g.=*Mytilus galloprovincialis*; A.b.=*Americamysis bahia*; E.e.=*Eohaustorius estuarius*; L.p.=*Leptocheirus plumulosus*; B.p.=*Brachionus plicatilis.*

4.2.3. Comparison of toxicity with SPME contaminant concentrations

Solid phase microextraction (SPME) fibers were used to estimate the relative concentration of organic contaminants (PAHs, PCBs, organochlorine pesticides) in splits of pore water samples used for toxicity testing. The SPME work was performed by Dr. Keith Maruya of the Southern California Coastal Water Research Program (SCCWRP), Long Beach, California.

Without conducting a toxicity identification evaluation (TIE), it might not be appropriate to assume that pore water concentrations of organic contaminants should correlate with toxicity, as other contaminant classes (e.g. metals) and non-contaminant factors (e.g. dissolved organic carbon, particulates) could influence bioavailability and subsequent toxicity. In addition, it would be unreasonable to expect all toxicity tests to result in similar effects due to species-specific sensitivities to contaminant and non-contaminant related factors that might contribute towards apparent toxicity.

It is interesting to note, however, that stations NS 11F and NS 14F, were typically ranked as both the most toxic and most contaminated by organics, based on SPME fiber analysis of pore water samples (Table 4-7). These two samples are located in historically the most contaminated areas of those sampled. It is unclear as to why the reference station SB90056 apparently had relatively high contamination by organics. Station SI21 is located well within Shelter Island, and was expected to be more impacted by metals than organics, which may explain the relatively low concentrations of organic contaminants detected using the SPME analysis, and the lack of agreement between the toxicity and SPME data for that sample (Figure 4-8).

Table 4-7. Summary preliminary organic chemical concentrations determined in pore water samples using solid phase micro-extraction.

Chem	Field	Vol tested	All (*n* ~100)	All (non-pyrethroids)		ΣPAH		ΣPCB		ΣOCP		Mean
Sample ID	Sample ID	(mL)	Mass (pg)	Rank	Mass (pg)	Rank	Mass (pg)	Rank	Mass (pg)	Rank	Mass (pg)	Rank	Rank[1]
SEAP-1	SI21	49	7	10	7	10	7	10	0	9	0	10	9.7
SEAP-2	SB2441	48	318	2	87	7	9	8	0	9	78	1	6.0
SEAP-3	SB90056	46	315	3	208	1	22	5	132	1	62	3	3.0
SEAP-4	Equip Blnk	43	204	4	94	6	14	7	75	2	11	9	6.0
SEAP-5	CP2243	28	90	8	85	8	9	8	42	5	33	5	6.0
SEAP-10	NS11-C	28	177	5	95	5	47	2	28	8	17	7	5.7
SEAP-11	NS14-C	28	77	9	66	9	21	6	40	6	16	8	6.7
NS11	NS11-F	28	127	7	127	3	43	3	46	3	38	4	3.3
NS14	NS14-F	27	128	6	111	4	57	1	45	4	18	6	3.7
NS24	NS24	67	394	1	140	2	36	4	36	7	68	2	4.3

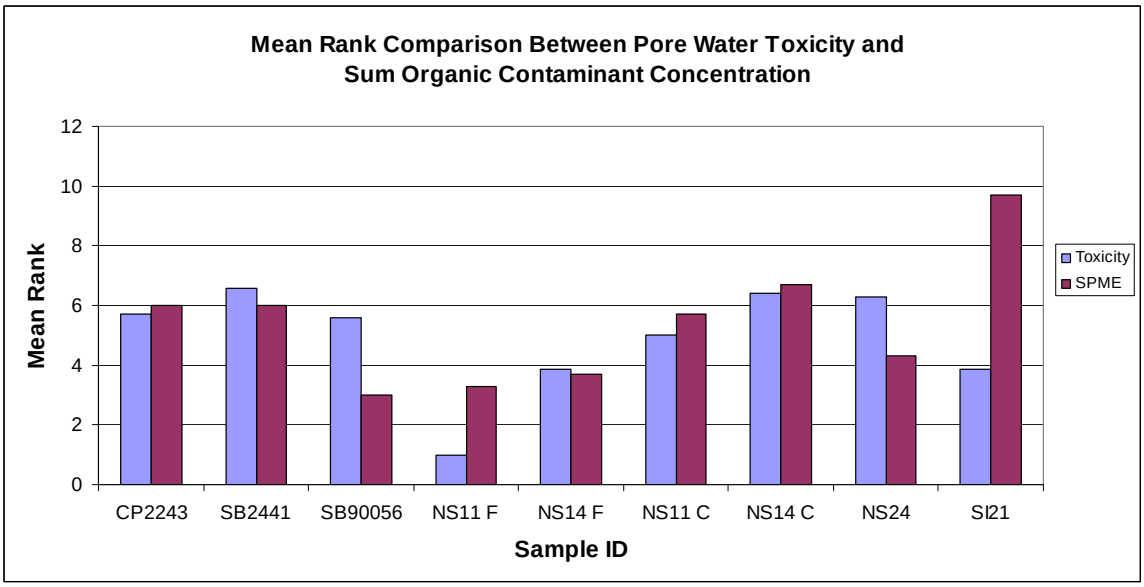

Figure 4-8. Mean ranks for all toxicity test methods employed and for all organic contaminant classes measured using solid phase microextraction (SPME) on splits of the same pore water samples.

4.2.4. Methods for SPME fiber analysis of pore water

Input from Dr. Keith Maruya (Southern California Coastal Water Research Project)

Solid phase microextraction (SPME) fibers coated with 7 µm polydimethylsiloxane (PDMS), purchased new from Supelco (Bellefonte, PA, USA), were conditioned at 320°C under a stream of ultrahigh purity helium in a GC injection port for 1 h and kept in a sealed glass vial in a freezer before use. PTFE-coated stir bars (13 × 3mm) and PTFE sheets were rinsed with deionized water, sonicated in methylene chloride (HRGC grade, Omnisolv, Gibbstown, NJ, USA) and dried at 100°C prior to use.

Ten porewater samples in their original vial (as shipped from SPAWAR) were allowed to come to room temperature (22±3°C) before being placed on a magnetic stir plate (Corning, NY, USA). After a PTFE stir bar was placed into the vial, a PTFE sheet was secured over the vial opening to minimize gas phase interactions. The sheet was then pierced by a SPME syringe assembly and the PDMS coated fiber extruded into the aqueous phase. Vial contents were agitated at 870 rpm and protected from ambient light by aluminum foil for a period of 119 h (~5 d).

Porewater exposed SPME fibers were analyzed using a Varian 3800 GC/Saturn 2000 ITMS (Varian, Walnut Creek, CA) with a 1079 split/splitless injector and an 8410 autosampler. The SPME syringe was manually injected into the injection port in splitless mode and the fiber thermally desorbed at 280°C for 6 min till split valve opening. The injector temperature was programmed from 100 to 280°C at ~100°C/min with a 20 minute hold time at the maximum temperature. Carrier gas was UHP helium with a flow rate of 1.0 mL/min. Chromatographic separation was achieved using a DB-5MS column (60m × 0.25mm × 0.25µm, J&W Scientific, Folsom, CA) temperature-programmed from 80°C (1 min hold) to 176°C at 8°C/min, followed by a ramp to 230°C at 1.5°C/min, and a final increase to 290°C at 5°C/min (29 minute hold). The temperatures of the ion trap, manifold and transfer line were 220, 120 and 280 °C, respectively. Mass spectra were acquired in the positive electron impact mode at 70 eV by selected ion storage (SIS) method.

Target analytes included 51 individual 2 to 6 ring PAH, 40 Cl_2-Cl_9 PCB congeners, 19 organochlorine pesticides (OCPs), 8 pyrethroids and 4 fipronil components and metabolites. External calibration was

performed for target analyte quantification using calibration solutions ranging between 50 to 2000 ppb. A mid-level calibration solution was analyzed periodically to monitor stability of instrument response. Mass sorbed by the SPME fiber (N_f) were reported for each analyte and were summed across each of the major analyte classes (e.g. PAH, PCBs, OCPs). Estimation of aqueous concentrations is not considered reliable due to lack of calibration constants (i.e. fiber-water partition coefficients) for the 119 h exposure period.

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5.0 EFFECTS OF CAGING ON ORGANISMS IN THE LABORATORY

Several experiments were conducted with the overall purpose of determining what kinds of exposure chambers would be suitable for the intended *in situ* test species, as identified by the literature review. Select results from this work were recently published as part of a leveraged effort with the NESDI program (Rosen and Lotufo 2010).

5.1. Caged Organisms in Laboratory Microcosm Exposures

The effects of caging on project specific *in situ* test organisms were evaluated in microcosm experiments in the laboratory. Leveraged with a Navy study designed to investigate the potential for effects associated with the simulated leakage of munitions constituents from discarded or unexploded ordnance, this experiment involved two species of amphipod (*Eohaustorius estuarius*, *Leptocheirus plumulosus*), one polychaete (*Neanthes arenaceodentata*), and embryos of the Mediterranean mussel (*Mytilus galloprovincialis*) (Figure 5-1). Amphipods and polychaetes were contained in exposure chambers modeled after Burton et al. (2005) (Figure 5-2). The *in situ* chambers were cylindrical and constructed of transparent core tubing of cellulose acetate butyrate or Eastman Tenite Butyrate with a 6.67-cm inner diameter (ID), 6.98-cm outer diameter (OD), 0.16-cm wall thickness, and cut to a length of 12.7 cm. Polyethylene closures capped each end. Two rectangular windows (4 X 8 cm) were cut on each core tube opposite each other and covered with nylon mesh (80 µm). Amphipods were loaded at a rate of 20 per chamber, while polychaetes were loaded at 5 per chamber. Mussel embryos (approximately 200 embryos < 4 h old) were contained in 20 ml glass scintillation vials outfitted with a 25 µm mesh cap (Figure 18, 20). The chamber design for the embryo-development tests was selected based on another set of experiments conducted as part of the SERDP project to optimize mussel exposure chamber design.

The experiment was conducted using uncontaminated sediment obtained from the amphipod (*E. estuarius*) collection site. This sediment is sandy in nature, and is typically used as a negative control for amphipod sediment toxicity tests. Contaminated treatments received two uniformly sized fragments of Composition B, with a total mass of 500 mg. Composition B is a military unique formulation containing 39.5% 2,4,6-trinitrotoluene (TNT), 59.5% hexahydro-1,3,5-Trinitro-1,3,5-Triazine (also known as Royal Demolition Explosive, or RDX), and 1% wax.. The Comp B fragments were placed either on the sediment surface or buried 1 cm below the surface of the sediment. Experiments were conducted using two different flow rates: a) no flow, and b) ½ turnover per day. Synthetic sea water (Instant Ocean) was used as the dilution water, which was mixed at a salinity of 30 ‰. Exposures spanned between 2 days (mussel embryos) and 10 days (amphipods, polychaetes). The test endpoint for the mussel tests was normal survival, while survival and bioaccumulation were the endpoints for the amphipods and polychaetes.

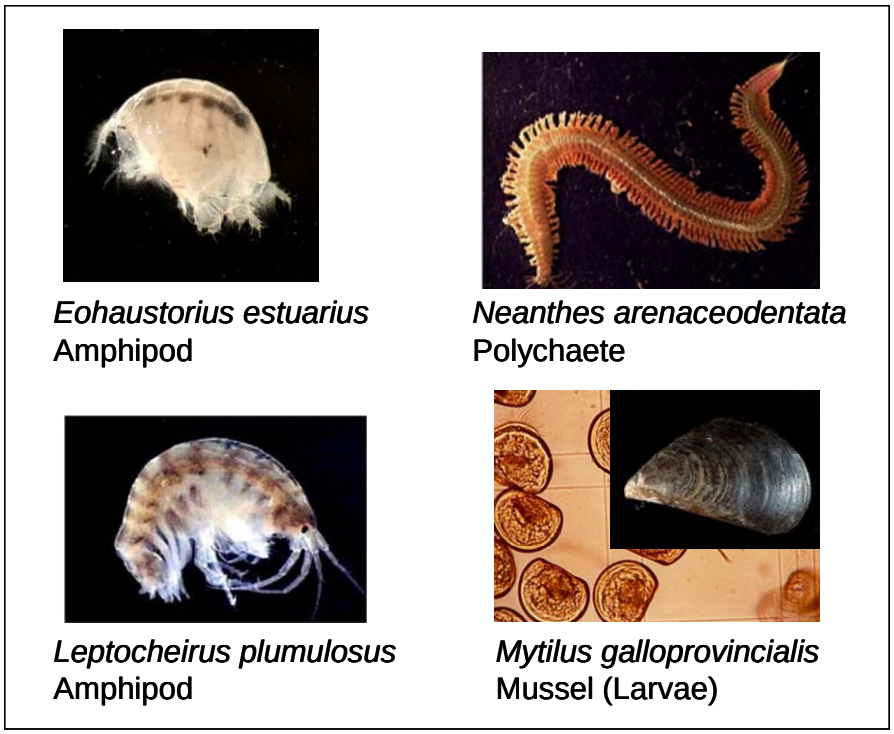

Figure 5-1. Estuarine test organisms selected for in situ bioassay development.

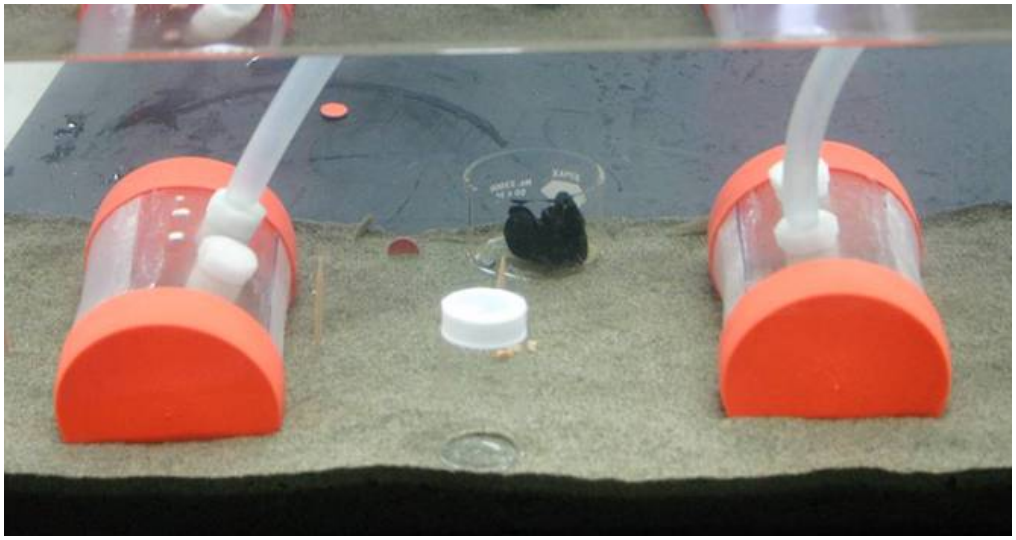

Figure 5-2. Test organism chambers housing marine organisms to assess the impacts of caging and response in spiked sediments. Amphipods (*E. estuarius, L. plumulosus*) and polychaetes (*N. arenaceodentata*) were housed in the larger chambers. Mussel (*M. galloprovincialis*) embryos were contained in the smaller vials with white mesh cap.

No significant mortality was observed under any exposure condition for either amphipod species or the polychaete (Figure 5-3). This suggests that these species were not negatively impacted by caging. The lack of effects in the Comp B exposures was not surprising considering that overlying and pore water concentrations did not approach previously determined toxic thresholds for TNT (or its transformation products) or RDX for these species. Sublethal concentrations of these contaminants, however, were

measured in the tissues, particularly under the worst case exposure scenario (Comp B fragment at sediment surface with no exchange of overlying water).

The successful performance of the *in situ* mussel embryo chambers was also illustrated in this experiment. Lethal and sublethal toxicity (expressed as a combined endpoint referred to as normal survival) were observed for mussel larvae under the worst case exposure scenario (Comp B fragment at sediment surface with no exchange of overlying water; Treatment SE in Figure 5-3). This was the case whether embryos were exposed *'in situ'* or when embryos were exposed to discrete samples removed from the overlying water using standard laboratory methods. The toxicity in this treatment was explained by the relatively high TNT concentrations measured in the microcosms during the exposure, which approached concentrations known to reduce normal larval development for this species (Rosen and Lotufo 2007). Although the TNT concentration inside the chambers was not measured (only the surrounding water), the fact that embryo toxicity was observed suggests that TNT concentrations inside the chamber did approach those outside, which was substantiated by the salinity equilibration experiments using these chambers.

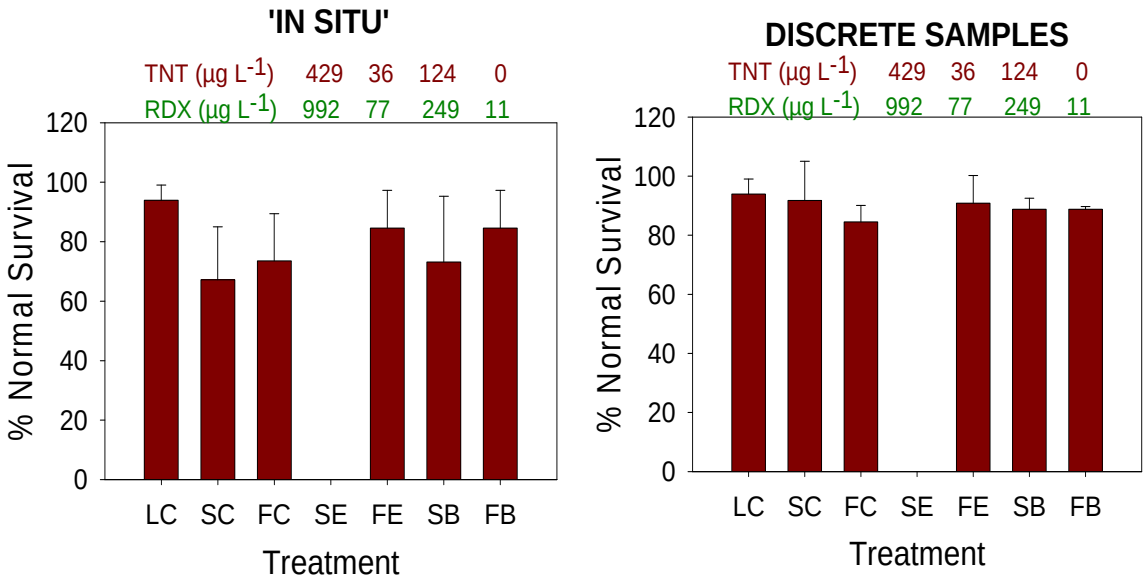

Figure 5-3. Mean percent normal survival (± 1 s.d.) from mussel (*Mytilus galloprovincialis*) embryo-larval development experiments conducted in experimental in situ chambers (modified scintillation vials) and using normal laboratory methods on discrete samples. Substantial toxicity was observed only in the worst case exposure scenario (SE), where TNT concentrations approach known thresholds for this endpoint. SE= Static conditions (no water exchange), Exposed Comp B. LC= Lab Control. SC= Static Control. FC=flow control. FE=Flow, Exposed. SB=Static, Buried Comp B. FB=Flow, Buried.

5.2. Chamber Design Development for Smaller Test Organisms- Mussel Embryo-Larval Development Tests

5.2.1. Chamber Design Exposure Comparison

It is likely that the fairly basic exposure chambers employed by Burton et al. (2005) and references therein, or fairly minor modifications of those chambers, will be acceptable for most of the test organisms used in this study. Smaller exposure chambers, however, will likely be required for smaller organisms. Mussel embryos require a smaller mesh size (e.g., 20-40 µm) due to their near microscopic size (60 µm at start of exposure) and can be lost if the chamber design requires multiple steps for recovery, consolidation, transfer, and preservation in vials for later microscopic analysis (Figure 5-4). Several

chamber designs were compared for their appropriateness for the mussel embryo-larval development assay. It is anticipated that other relevant smaller test species (e.g., rotifers, dinoflagellates) could be exposed in these small scale chambers as well.

Figure 5-4. Examples of exposure chamber designs evaluated for mussel embryo-larval development testing. Drum design for smaller organisms (top left) from Phillips et al. (2004). Simple chamber for larger invertebrates (bottom right) based on those by Burton et al. (2004).

Exposure chambers evaluated included:

a) Drum design (Anderson et al., 1998; Phillips et al. 2006) consisting of relatively large mesh area (44%);
b) 20 ml glass scintillation vials with mesh caps;
c) 20 ml glass shell vials with mesh caps;
d) Standard in situ chambers outfitted with 20 µm mesh (Burton et al. 2005);
e) 8 oz. high density polyethylene (HDPE) cups with lids with 3" diameter mesh cutouts.

All exposure chamber designs utilized 20 µm polyethylene mesh for water exchange.

Chambers were evaluated concurrently with the same batch of mussel embryos. Each design was exposed in triplicate, both in the laboratory under standard testing conditions for this species (15 °C, 30 ‰; USEPA 1995) and off the SPAWAR research pier at a depth of ~1 m.

Recoveries of normally developed larvae from the Drum design were the poorest, while modified scintillation and shell vials resulted in the best recoveries in both the lab and field (Figure 5-5). While the Burton et al. (2005) chambers performed fairly well in the lab, lower recoveries of normal larvae were observed n the field. It is unclear as to what specifically caused these discrepancies, however, it is suggested that those chambers that require more transfer steps to consolidate larvae resulted in the

greatest loss. Scintillation and shell vials could be exposed, preserved, and counted under the microscope in the same container, eliminating the need for transfer.

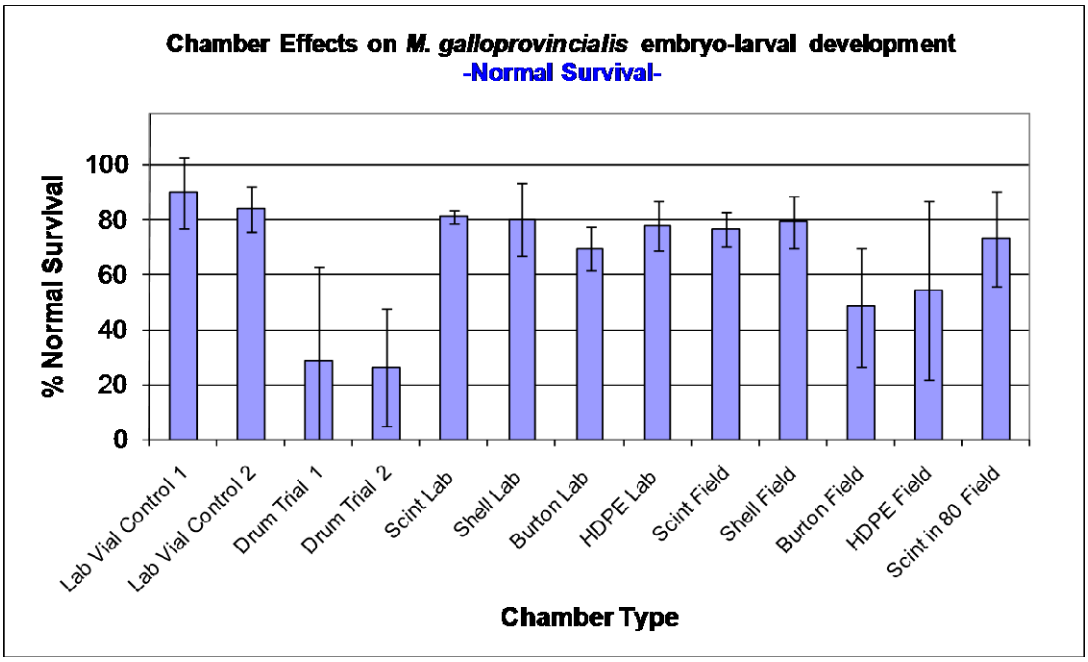

Figure 5-5. Chamber effects on mussel embryo-larval development.

5.2.2. Chamber Equilibration Study

The successful use of the modified scintillation vial chamber design resulted in the desire to assess the rate of equilibration between conditions outside the chamber and inside the chamber. This was of particular interest for these chambers due to their relatively small mesh size (20 µm) and small screen area. A simple experiment using salinity as a means of estimating equilibrium conditions was conducted (Figure 5-6). A 20 L plastic tank was filled with natural seawater (33 ‰). Subsequently, 27 chambers were filled with deionized water (0 ‰) and placed on their sides on the bottom of the tank. Three replicate vials were removed at 9 different sampling points over a period of approximately 24 hours, and salinity of the contents measured with an Orion Model 105 Salinity/Conductivity Meter. The experiment was conducted under two flow conditions: static and under a continuous flow rate of 100 GPH using a MarineLand Bio-Wheel Pro30 aquarium filter.

Figure 5-6. Freshwater-containing mussel chambers during salinity equilibration study.

Salinity within the vials rapidly increased during the first few hours of the exposure (Figure 5-7). Within 4 hours, salinity in the vials was 79 and 88% of the salinity in the external environment for static and flow through conditions, respectively. Steady-state conditions were achieved within 6 hours under the flow conditions, and by the 18 hour time-point under static conditions. It is expected that similar studies with salinity or dye will continue to be conducted as cage design throughout the project continues to develop.

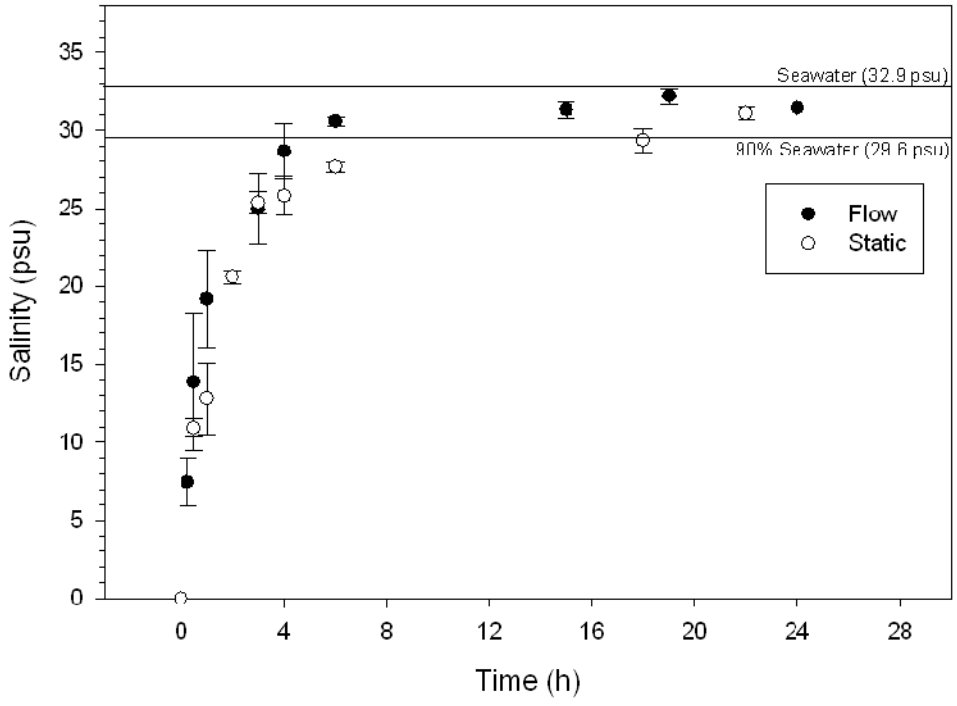

Figure 5-7. Salinity equilibration experiment results using prototype mussel embryo exposure chambers (modified scintillation vials with 20 µm mesh caps).

5.3. Shaker Experiments

To address inter-species sensitivity to physical stress that might be encountered either during transport to the field or while in the field, laboratory shaking experiments were conducted with select species. Experiments were conducted using Lab-Line Model 3520 Orbit Shakers set at three different speeds: 0 (control), 100, and 150 RPM. Testing was conducted in at least 2 *in situ* chamber types suitable for the specific test species. *In situ* chambers which were held in 400 mL glass beakers containing 200 ml uncontaminated, filtered (0.45 µm) natural seawater (30 ‰). Air was not removed from the top of the chambers, as chambers were not completely submersed in water. Therefore, it is expected that this exposure scenario represents worst-case conditions. All experiments were conducted at 20 °C. For comparison, *in situ* chambers containing two of the species were also deployed off the research pier at SPAWAR, at a depth of 1 m, where conditions were fairly calm through the duration of the exposure. *In situ* exposures were conducted at a temperature of ~16 °C and salinity of 33 ‰ (measured at exposure termination only).

Three short-list test species were employed in the initial experiment: 5 mm amphipods (*Leptocheirus plumulosus*), juvenile mysid shrimp (*Americamysis bahia*), and mussel (*Mytilus galloprovincialis*) embryos. All species were acclimated to test conditions over a 3 day period prior to experiment initiation. Mussel embryos were obtained by heat shocking conditioned adult mussels to initiate spawning, and added to experimental chambers within 4 h of fertilization. A total of ~200 embryos were added to each mussel embryo chamber. Mussel chambers were either a) preconditioned 20 mL glass scintillation vials with plastic screw caps that were modified with 20 µm Nitex mesh screen covering an opening with a diameter of approximately 1.5 cm, or b) large chambers used by Burton et al. (2005) with a mesh size of 20 µm. Amphipods and mysids were held in both larger chambers similar to those used by Burton et al. (2005) with a mesh size of 74µm, and smaller experimental chambers with a mesh size of 250 µm. Ten amphipods and ten mysids were added to each of the chambers. All lab testing was conducted in replicates of 3, while *in situ* exposures were limited to two replicates. Fresh *Artemia* nauplii were fed to each of the mysid/amphipod chambers once per day. Experiments were conducted without any renewal for a period of 48 h. Results are presented in Figure 5-8 through Figure 5-10.

Shaker Results and Conclusions:

Large Chambers

Amphipods and mysids both faired quite well in the Large chambers at 100 RPM after 48 h, but had reduced survival (at 48 h only) at 150 RPM. The reduced survival at 150 RPM is expected to only be statistically significant for mysids. Amphipod survival was only slightly reduced (mean survival = 70 ± 17%), while mysids were dramatically affected (mean survival = 3.3 ± 5.8%). Amphipods, which are considerably larger than mysids, may have avoided being tossed around as much, and were observed to be near the bottom of the containers during exposure.

Small Chambers

The Small chambers resulted in relatively little impacts on survival for both species at 100 RPM, but survival for both species was reduced at 150 RPM. Due to some variability in amphipod controls exposed in Small chambers, the amphipod reduction in survival may not be statistically significant. Mysid survival, however, will likely be statistically lower under the highest shaking speed, once again suggesting that the amphipods are more tolerant of higher degrees of physical stress.

In Situ Chambers

Both amphipods and mysids had high survival rates in the 48 h field deployments off the SPAWAR Pier in both chamber sizes. Mysid survival was slightly lower (mean = 80%) in the small chambers, but low replication for the field deployments was too low to make such a conclusion.

Mussel embryos

Mussel embryo-larval development results are generally inconclusive. Although lab controls did well, the scintillation vial in situ controls resulted in relatively poor recoveries of normal larvae. This makes interpretation of the scintillation vial data difficult, especially due to the lack of a trend (150 RPM chambers had higher recoveries than did 100 RPM chambers). Large chambers (Burton et al. 2005) resulted in good performance under static conditions, but essentially no normal development at both 100 and 150 RPM. It should be pointed out that the exposure scenario for the Burton chambers in this study design (open at top, with lots of turbulence) is unlikely to ever be experienced by mussel embryos enclosed in a water-filled chamber with a relatively small mesh size, reducing flow rate. This experiment could be repeated if so desired.

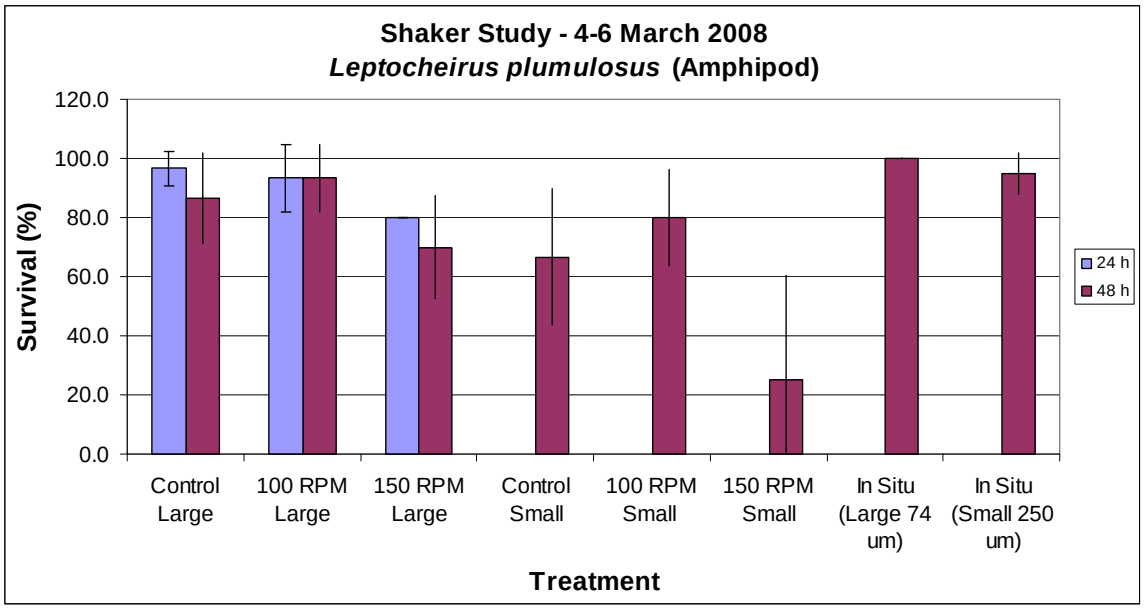

Figure 5-8. Shaker study with *Leptocheirus plumulosus*.

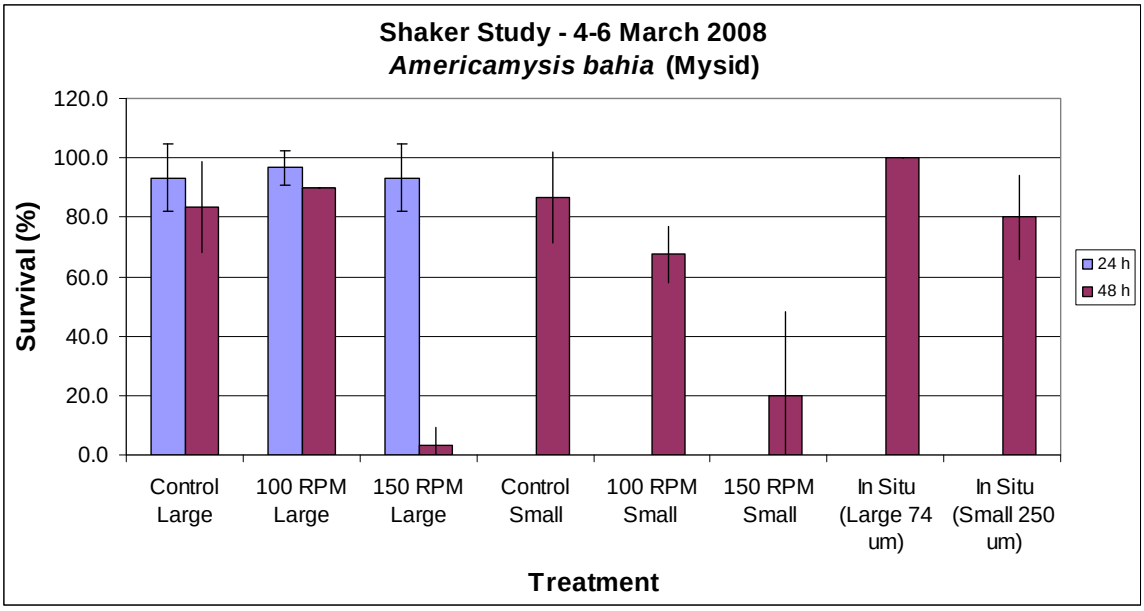

Figure 5-9. Shaker study with *Americamysis bahia*.

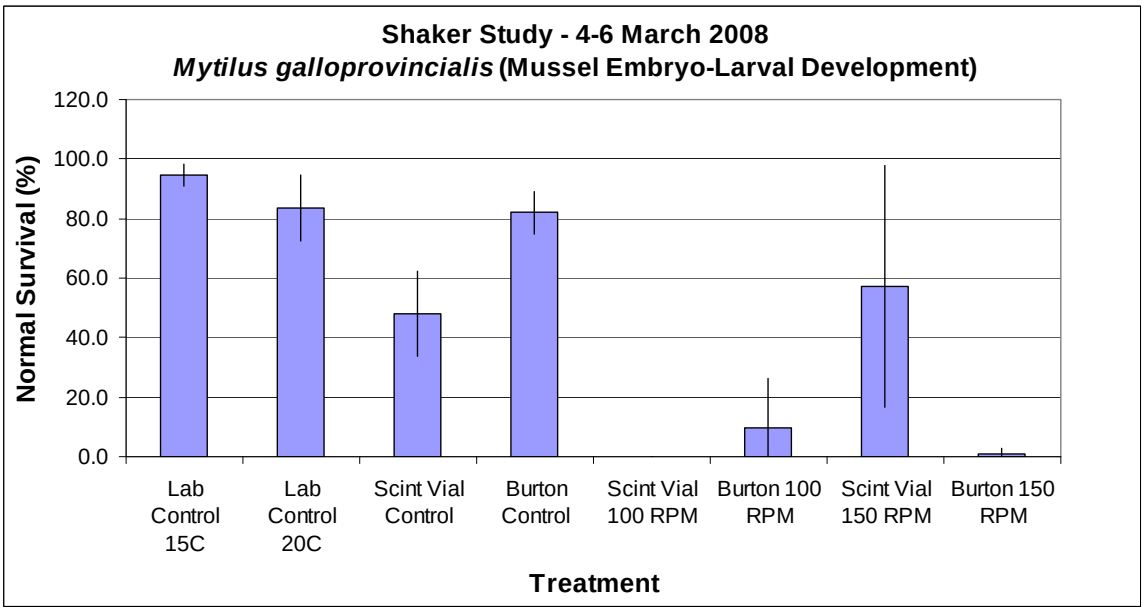

Figure 5-10. Shaker study with *Mytilus galloprovincialis*.

5.4. References

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6.0 DEVELOPMENT AND REFINEMENT OF NEW AND EXISTING TOOLS

As part of the initial SEAP approach, laboratory bioassays are conducted on many pore water samples freshly collected from the test site. One of the potentially most useful assays is the QwikLite assay, due to its speed and sensitivity; therefore additional testing was performed to better evaluate this bioassay.

6.1. Sensitivity Evaluation of New Commercial QwikLite Technology

QwikLite is a Navy-developed technology that measures light reduction from bioluminescent dinoflagellates as a measure of toxicity, providing a cost effective and rapid alternative or supplement to traditional bioassays. The test typically involves a 24 h exposure of the dinoflagellates to an aqueous sample, followed by controlled mechanical stimulation of the cells during the peak portion of their dark phase (when light output is greatest) under a 12h light: 12 dark photoperiod. The tool met most criteria identified by this project as a useful screening-level toxicity test, based mostly on knowledge of the Navy-derived version. A commercial version of QwikLite is currently being produced by Assure Controls, Inc., Vista, California (www.assurecontrols.com). Among other changes, the new unit uses a photodiode instead of a photomultiplier tube to quantify light production. The new test unit (QwikLite 200) was tested with copper and ammonia to confirm its relevance as a screening tool for pore waters.

All testing utilized the cosmopolitan bioluminescent dinoflagellate *Pyrocystis lunula*. This species is currently the species of choice with the QwikLite 200 test unit (Assure Controls 2007), but *Ceratocorys horrida* was also evaluated for comparative purposes (Figure 6-1). The guidance suggests an optimal temperature range of 17-27°C, and optimal salinity range of 30-35‰.

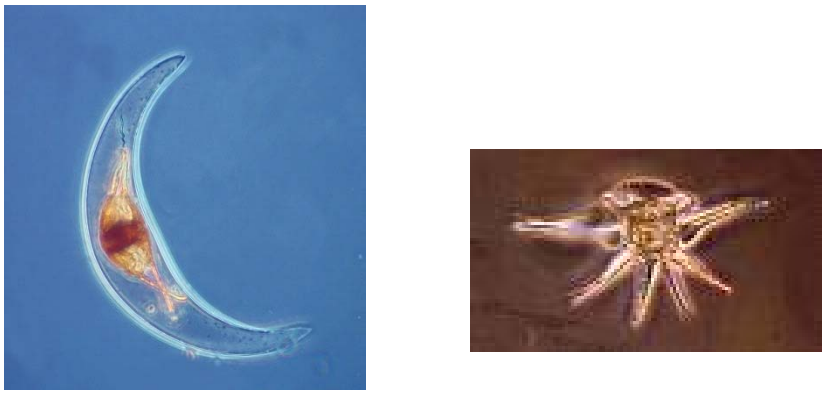

Figure 6-1. Two bioluminescent dinoflagellates that can be used in the QwikLite test sytem, *Pyrocystis lunula* (left) and *Ceratocorys horrida* (right).

P. lunula cultures were received from the vendor and used within 24 h of arrival. *C. horrida* was cultured at the SPAWAR bioassay laboratory. Cultures within 2 weeks of creation with fresh culture media were used for the experiments. Six concentrations of each toxicant were prepared for each test. Six replicates were tested for each concentration. EC50 values were calculated using the QwikLite 200 software, which uses the linear interpolation method (Table 6-1).

Table 6-1. Median effects concentrations (EC50) for two toxicants using the QwikLite 200 toxicity test system. EC50 values are provided for two different dinoflagellate species, *Pyrocystis lunula* and *Ceratocorys horrida*.

	EC50 (mg L^{-1})	
Species	Copper	Un-ionized Ammonia
P. lunula	0.126	0.706
C. horrida	0.308	0.192

The results of these experiments indicated that sensitivity was similar to that previously reported using the Navy-derived test unit (Lapota et al. 2007, Rosen et al. 2008, Stauber et al. 2008). Copper sensitivity for *P. lunula* was also similar (within a factor of 2) to that reported by others where the endpoint was re-establishment of bioluminescence following light depletion (Craig et al. 2003, Heimann et al. 2002). In addition EC50 values suggested similar sensitivity to copper between the two species, but ammonia was less toxic to *P. lunula* by nearly a factor of 4. A reduced sensitivity to ammonia is preferable for pore water screening, because it can lead to false positive conclusions when detection of contamination associated with other chemical classes is desired. In addition, it became quite apparent that *P. lunula* better tolerated handling and physical stress associated with transportation than did *C. horrida*. *C. horrida* did not travel well from laboratory to laboratory during our collaborative work with Assure Controls, requires more culture maintenance (e.g. more frequent renewal of exposure media due to higher growth rates), and is more likely to emit light prematurely due to minor agitation as cuvettes were loaded into the testing unit.

This testing established the viability of the new unit, where little variation within treatments was observed. The repeatability of the test was confirmed using different *P. lunula* cultures at the Wright State University lab during the salinity/temperature experiments, where a reduction in light output was also reduced using a nominal copper concentration of 125 µg/L. *P. lunula* also appeared to result in a lower risk for false positives associated with ammonia in pore water samples at Naval Station San Diego, simplifying data interpretation with this species as compared to *C. horrida*.

It is anticipated that *P. lunula* will be used *in situ* where relevant. Exposure chamber considerations for dinoflagellates are currently under investigation.

6.2. Method Development for Polychaete Post-Exposure Feeding Rate Assay

Post exposure feeding rate of the polychaete *Neanthes arenaceodentata* (Figure 6-2) was investigated as part of this project as a rapid sediment bioassay that can be conducted either in the laboratory or the field. Successful demonstration of this tool could fill a niche for short term, sublethal *in situ* sediment toxicity testing. Traditional toxicity testing with this species may not be appropriate *in situ* as standard endpoints are based on long-term survival and growth (USEPA and USACE, 1998). The utility of a growth endpoint in field exposures may be problematic due to the differences in food quality and quantity at different sites and the fact that feeding specified rations to field organisms (as is done in laboratory testing) might be logistically challenging. In addition, significant growth requires relatively long exposure times for polychaetes.

Moreira et al. (2005) reported success with postexposure feeding rate using a European polychaete, *Hediste (Nereis) diversicolor.* The endpoint involves observations of polychaete feeding rate on *Artemia* (brine shrimp) nauplii for 1 hr following a 48 h exposure period in surficial sediment. Feeding rate was also substantially more sensitive than survival in laboratory exposures to copper (Moreira et al. 2005). Because temperature and salinity affected feeding rate on *H. diversicolor*, regression equations were developed to derive “adjusted” feeding rates that factor in these parameters for better interpretation of resulting data. A manuscript detailing the development of this assay using *N. arenaceodentata* is provided in Section 7.0.

Figure 6-2. Photographs of *Neanthes arenaceodentata* adult (main picture) and *Artemia* sp. nauplii (smaller picture).

6.3. References

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7.0 DEVELOPMENT OF A POST-EXPOSURE FEEDING RATE ASSAY USING THE MARINE POLYCHAETE *NEANTHES ARENACEODENTATA* SUITABLE FOR LABORATORY AND *IN SITU* EXPOSURES *(submitted to Environmental Toxicology & Chemistry)*

7.1. Overview

This study examined the suitability for the use of the polychaetous annelid *Neanthes arenaceodentata* in a short-term sub-lethal bioassay based on post exposure feeding rate. Quantification of feeding rate was determined by a 1-h post exposure feeding period to *Artemia franciscana* nauplii following a 48-h aqueous exposure period. Both lethality and feeding rate were assessed following exposure to copper and phenanthrene, with the copper results being compared to those available from similar studies that used the polychaete *Hediste diversicolor*. Laboratory assessment on the effect of varying two common variables in estuarine environments, temperature and salinity, on post exposure feeding to both clean and copper spiked seawater samples was also conducted. The 48- and 96-h LC50s for copper were 156 and 80 µg/L, respectively, while the 48 h EC50 determine by feeding rate was 57 µg/L. The 48 h LC50 for phenanthrene was 2,224 µg/L while the 48 h feeding rate EC50 was 345 µg/L (more sensitive by a factor of greater than six). The sensitivity of the post exposure feeding rate endpoint to two representative chemicals that are frequently elevated in contaminated sediments, rapid exposure time, ecological relevance, and relatively simple approach with *N. arenaceodentata*, suggest that this assay has potential for use as a tool for sub-lethal effects assessment, with particular promise for *in situ* applications. The utility of this assay in actual marine and estuarine sediments is currently being assessed *in situ* at several North American sites, and will be reported in future publications.

7.2. Introduction

Anthropogenic contamination of coastal waters has been a long standing problem that has been a cornerstone in policy making by environmental agencies. Traditional methods for evaluating sediment toxicity, one of several means of assessing sediment quality for decision making in regulatory programs, focus on endpoints such as survival, growth and reproduction (EPA, 1994). These methods are generally well developed for the laboratory, but are relatively undeveloped for use in *in situ* (i.e. field) toxicity testing, particularly with respect to marine endpoints (Rosen et al. 2009). *In situ* toxicity tests have the distinct advantage over the former in that they have the potential to provide more accurate assessment of exposure and effects in the field, thus improving the accuracy of management decisions. Detailed accounts of these advantages are provided elsewhere (e.g. Anderson et al. 2004, Burton et al. 2005, Liber et al. 2007, Salazar and Salazar 2007, Wharfe et al. 2007, Rosen et al. 2009), but include reduction of artifacts associated with sample manipulation/extraction, exposure under unrealistic (i.e. static) conditions, and the ability to include time-varying stressors and other site-specific conditions in the assessment.

Recent *in situ* marine toxicity testing has involved commonly used and readily available laboratory test organisms including amphipods (Kater et al. 2001, Anderson et al. 2004), clams (Ringwood and Keppler 2002), mysids (Clark et al. 1987), mussel (Anderson et al. 1998, Geffard et al. 2001, Katz and Rosen 2005) and sea urchin (Beiras et al. 2001) embryos, and polychaetes (Moreira et al. 2005). These tests involve both acute and chronic/sub-lethal endpoints, and show promise as reliable indicators of sediment or aqueous toxicity. There is a continued need, however, for the identification and implementation of easily used and understood sub-lethal endpoints that can be assessed within reasonable timeframes and cost.

Post exposure feeding rate has recently been explored as a sub-lethal endpoint with great potential for use *in situ* (Maltby et al. 2002, McWilliam and Baird 2002, Castro et al. 2004, Moreira et al. 2005, Moreira et al. 2006). These assays typically involve exposure of test organisms to a specified quantity of food for a

brief period (e.g. 1 h) following a short-term (e.g. 48 h) laboratory or field exposure. The short-term required to observe effects on feeding rate and the ecological relevancy of the endpoint (a reduced ability to feed can have longer-term implications on growth, reproduction, and survival, and hence population and community structure; Moreira et al. 2005, and references therein) make these methods attractive for use in the field.

Moreira et al. (2005) describe the use of the European estuarine polychaete *Hediste diversicolor* as a test organism in a post exposure feeding rate assay. The current study sought to develop a similar approach using the cosmopolitan marine polychaete *Neanthes arenaceodentata*, which could expand the geographical use of such a test as this species is widespread on both coasts of the United States, the Atlantic coasts of Spain and France and the Pacific (Pesch and Schauer, 1988; ASTM 2000). The development of a post exposure feeding rate assay using *N. arenaceodentata*, therefore, potentially provides a valuable addition to the battery of existing marine toxicity test methods that can be employed to assess contaminant impact on environmental health.

7.3. Methods

Assay overview

The primary objective of this study was to determine if post exposure feeding rate is a viable test endpoint with *N. arenaceodentata*, thereby providing an alternative tool for developing rapid, sensitive, reproducible, and sub-lethal toxicity data in both laboratory and field applications. The approach involved assessment of the relative sensitivities of *N. arenaceodentata* to select compounds (copper and phenanthrene) under controlled laboratory conditions, as well as to manipulation of factors that frequently vary in marine and estuarine environments (salinity and temperature). The proposed method is a short-term (48-96 h) exposure that can be used to identify potential contamination in surficial sediments, at the sediment-water interface, in the water column, or in contaminated discharges. The testing approach is similar to the 48 h method using *H. diversicolor* developed by Moreira et al. (2005), which provided us the ability to make comparisons between the two species.

Although it is expected that testing can be conducted in multiple matrices, the experiments described below were performed in water only exposures. Investigations at contaminated sediment sites *in situ* are currently underway (data not shown).

Assay organism

N. arenaceodentata and related Nereid polychaetes have been used extensively in North America for acute and chronic toxicity (Johns et al. 1990, Emery and Dillon 1996, Reish et al. 1999, ASTM 2000) and bioaccumulation testing (Lee et al, 2001), and they continue to be the subject of novel test development. Several summaries exist that focus either exclusively on the use of *N. arenaceodentata* as a test organism or reference them heavily (Reish et al, 1976; Reish and Gerlinger, 1984; Johns et al, 1990, Reish and Gerlinger 1997). Dillon et al (1993) provide a comprehensive evaluation on the use of *N. arenaceodentata* in a sub-lethal chronic survival and growth bioassay, which includes the effects of sediment type, water conditions (e.g. ammonia and dissolved oxygen concentrations), salinity, and cadmium as a reference toxicant. Additionally, there exists considerable data on *N. arenaceodentata* sensitivity to common environmental pollutants (Reish and Gerlinger 1997, and references therein). More recently, the survival and growth test endpoints using standard methods (ASTM 2000) have been suggested as preferred endpoints for use in newly developed California Sediment Quality Objectives (SQOs; Bay et al. 2007).

N. arenaceodentata can be cultured relatively easily when compared to other common test species (Pesch and Schauer, 1988; Dillon et al, 1993) or can be purchased directly from an established long-term population cultured by Dr. Don Reish of California State University Long Beach (CSULB). The worms attained from the CSULB cultures are of similar size and life stage. Additionally, since the worms come

from the same source, they represent a homogenous population that should ensure reduced variability in stressor response.

Most worms used in this study were purchased from CSULB and acclimated to test conditions at SSC Pacific for a minimum of 2-3 days prior to test initiation. In some cases, the second or third generations of the CSULB worms were used following long-term maintenance at the SSC Pacific bioassay lab. Test organisms were approximately 6 weeks of age (~2 mg dry wt. each) at test initiation. The survival and growth method (ASTM 2000) calls for use of emergent juveniles (EJs) approximately 2 weeks of age at test initiation. We found that the smaller worms were more variable in terms of ability to feed on *Artemia* nauplii in initial trials in comparison to adult (6 week) worms. This could be due to their substantially smaller size (~0.3 mg dry wt. each) as EJs. During holding, worms fed on the green algae *Enteromorpha sp*. that was provided by the culturing lab.

Feeding rate assay preparation

Borosilicate glass scintillation vials (20 mL) were used for the feeding rate portion of the assay. At least 72 h prior to this, vials were conditioned for 24 h in uncontaminated 0.45 µm flowing seawater, rinsed in deionized water, and allowed to air dry to reduce any contaminants that might leach from the glass itself. The *Artemia* nauplii were concurrently hatched from cysts (San Francisco Bay Brand) in clean seawater at 25 ºC. Density of the nauplii (24 h old) was then determined by placing an aliquot of the well-mixed suspension on a slide and fixing the sample in 10% buffered formalin. The density of the suspension was adjusted by adding seawater so that ~120 nauplii (typically more than can be consumed by one worm in an hour) could be introduced to each vial by pipetting 500-1000 µL into each vial.

The appropriate volume of the nauplii suspension was then distributed to each clean vial and frozen at -20 ºC. Random subsets of 10 vials were subsequently counted prior to the tests to ensure that the nauplii density was within the range targeted and did not vary by more than 10%. The preparation of the feeding vials is highly important because the starting number of *Artemia* in each vial must be as consistent as possible to reduce possible bias.

Organism exposure

Test conditions are summarized in Table 7-1. Where relevant, these are similar to those developed for survival and growth tests with *N. arenaceodentata* (ASTM 2000). Test solutions were made as described below for each toxicant. Exposure vessels were 300 mL glass beakers that contained 250 mL of aqueous exposure media. Each test concentration was replicated four times, with five individuals added to each replicate. Ambient laboratory lighting was used under a 16 h light: 8 h dark photoperiod. Test organisms were not fed during the exposure portion of the assay. Daily measurement of pH, salinity, temperature, and dissolved oxygen (D.O.) were made throughout the exposure period. For *in situ* exposures, it is recommended that water quality be recorded at exposure initiation and termination, and ideally continuously using field deployable logging devices (i.e. HydroLab Datasondes, In Situ, Inc. Troll instruments) positioned inside a representative test chamber (Rosen et al. 2009).

Copper experiment

Copper was used as a representative of metal toxicity due to its relatively high toxicity and the large body of literature examining copper effects on *N. arenaceodentata* in established test protocols (Reish and Gerlinger 1997). Test concentrations were 0, 25, 50, 100 and 200 µg/L (0.5 dilution factor). The range selected bracketed the majority of reported 96 h LC50 values (range from 77 to 570 µg/L; Reish and Gerlinger, 1997). One 96 h (mortality recorded only) static-renewal exposure (renewal at 48 h) was conducted, and one 48 h (mortality and post exposure feeding rate) static exposure were conducted. Test solutions were made from filtered seawater and reagent grade copper (II) sulfate pentahydrate ($CuSO_4 \bullet 5H_2O$). For the 48 h experiments, samples were analyzed in-house via graphite furnace atomic absorption spectrometry using methods described by Rivera et al. (2005).

Table 7-1. Summary of test conditions for post exposure feeding rate assay with *N. arenaceodentata*.

Parameter	Description
Test type:	Static non-renewal (lab) Static renewal (lab) *In situ* exposure conditions (field)
Salinity	34 ± 2 ‰
Temperature	20 ± 1 ºC
Light quality	Ambient laboratory illumination (lab) Ambient field conditions (field)
Photoperiod	Sample exposure: 16 h light: 8 h dark (lab) Post exposure feeding: complete darkness
Test chamber size	Sample exposure: *in situ* chamber or 300 mL beaker Post exposure feeding: 20 mL scintillation vial
Test solution volume:	Sample exposure: 250 mL Post exposure feeding: 3 mL filtered seawater
Age of test organism	6 weeks
No. replicate chambers/concentration	4
No. organisms/replicate	Sample/field exposure: 10 Post exposure feeding: 1
Source of food	Newly hatched *Artemia* nauplii
Feeding regime	Sample exposure: None Post exposure feeding: Access to 120 freshly hatched, previously frozen Artemia nauplii
Dilution water	Uncontaminated 0.45 µm filtered natural seawater
Test concentrations	Minimum of 4 and a control for dilution series testing
Test duration	Sample/field exposure: 48 or 96 h Post exposure feeding: 1 h
Endpoint	Feeding rate (# nauplii consumed per hour)
Test acceptability criteria	90% survival in lab controls At least 70 *Artemia* nauplii consumed in 1 h in controls

Phenanthrene experiment

Polycyclic aromatic hydrocarbons (PAH) are also common contaminants of concern in sediments, and phenanthrene tends to be among the most toxic to marine invertebrates (Neff 1979, Emery and Dillon 1996). Stock solutions were made by weighing out solid phenanthrene (Argos Organics, Fischer Scientific, 97% purity) into a glass scintillation vial, which was dissolved with pesticide grade acetone A 96 h range finding experiment with concentrations 0, 100, 200, 500, 1000 and 2000 µg/L that included lethality only was conducted under static conditions, with a single renewal at 48 h. The subsequent 48 h

definitive experiment included both survival and post exposure feeding rate endpoints. The exposure concentrations for this experiment followed a 0.6 dilution factor (0, 500, 860, 1400, 2400, 4000 µg/L phenanthrene), with a renewal with fresh test solutions at 24 hours. The maximum concentration of acetone in test solutions was 1 ml/L. Chemical analysis on the exposure solutions was conducted using GC-MS with a reporting limit of 0.5 µg/L. Measured concentrations were expressed as the average of the initial and 24 hour (prior to renewal) concentration.

Temperature and Salinity Effects

Three temperatures and salinities (for a total of nine combined treatments) were evaluated concurrently to determine the effects of varying temperature and salinity on the response of *N. arenaceodentata* in the feeding rate bioassay. Each temperature/salinity combination was tested in uncontaminated seawater as well as in a copper-spiked test solution at a concentration (80 µg/L) where effects would be expected to occur. The spiked copper experiment was used to examine any effects that temperature and salinity might have on representative contaminant-specific sensitivity of the test endpoint during potential field exposures.

Temperature was held at 15, 20, or 25°C, and salinity was held at 20, 27, or 34 ‰. Uncontaminated seawater was filtered with 0.45 µm nitrocellulose membrane filters and the different test salinities were created by diluting filtered seawater with 18 M ohm E-pure water. Due to the limited numbers of worms available for this experiment, three replicates were used for each treatment instead of four. Worms were maintained under normal (control) conditions (20 °C, 34 ‰) until test initiation, but were held under the manipulated condition during both the 48 h solution exposure and 1 h post exposure feeding rate phases of the experiment. The test animals were not acclimated to test conditions since deployment at field sites would also expose test animals to a rapid change in conditions.

Feeding quantification

Prior to starting each experiment, vials were acclimated to the testing temperature and filtered seawater added to bring the total volume to 3 mL. We observed that larger seawater volumes sometimes resulted in a reduced interaction between the worm (sometimes seen adhered to the inside of the glass at the water line) and the food on the bottom of the vial. Reduction to the 3 mL water volume increased interaction and reduced feeding variability among replicates in preliminary trials.

Upon termination of the test solution exposure, final mortality and behavioral observations were made. The post exposure feeding portion of the assay was then initiated by selecting three surviving worms of most consistent size from each beaker for transfer to individual feeding vials. Additionally, several surrogate control vials (also containing one worm each) were prepared a few minutes before the others in order to monitor the consumption rate, and prevent total consumption of *Artemia* by the worms. (In our study, one worm could typically consume 80-100 nauplii in one hour). Feeding start time was recorded for each group of vials, which were then placed into a darkened incubator. After documentation that surrogate vials had consumed the majority (but not all) of the nauplii, test vials were sequentially removed, end time recorded, and buffered formalin added to terminate feeding and preserve worms and *Artemia*. Final counts of remaining *Artemia* were made using an inverted microscope at 40x magnification. The number remaining from each vial was then converted to consumption rate per hour.

Data analysis

For the feeding rate experiments, the mean number of nauplii consumed per hour for each of the three worms removed from a test beaker was calculated, providing one value for each replicate. For the dose response studies, maximum likelihood probit analysis was performed using Toxcalc 5.0 (Tidepool Scientific) to generate LC50 and EC50 values. Prior to analysis, the data were arcsine square root transformed in Toxcalc to homogenize the variances. No observable effect concentrations (NOEC) and lowest observed effect concentrations (LOEC) were calculated using Wilcoxon Rank-Sum test

and Dunnett's test. For other comparisons, significant reduction from control survival and feeding rate was generated by t-tests using one tailed distribution and unequal variance. Both measured and nominal values were statistically analyzed. For copper, measured values were calculated by averaging chemically measured initial and final concentrations. Phenanthrene measured values were calculated by averaging initial and previous to renewal at 24 hours measured by chemical analysis.

7.4. Results

Copper experiments

The dose-response relationship of *N. arenaceodentata* exposure to measured copper concentrations is shown in Figure 7-1. Toxicity metrics and chemical analysis of test solutions are shown in Table 7-2 and Table 7-3, respectively. The 48 h feeding rate metrics (EC50, NOEC, LOEC) were all lower than the concurrent 48 h metrics, by a factor of approximately three. Based on nominal concentrations, the 96 h LC50 was approximately half the 48 h LC50. Chemical analysis (Table 7-3) showed that the levels of copper were only slightly lower (average=16-24%) than nominal levels, but remained relatively stable (a decline of about 15%) over the test period.

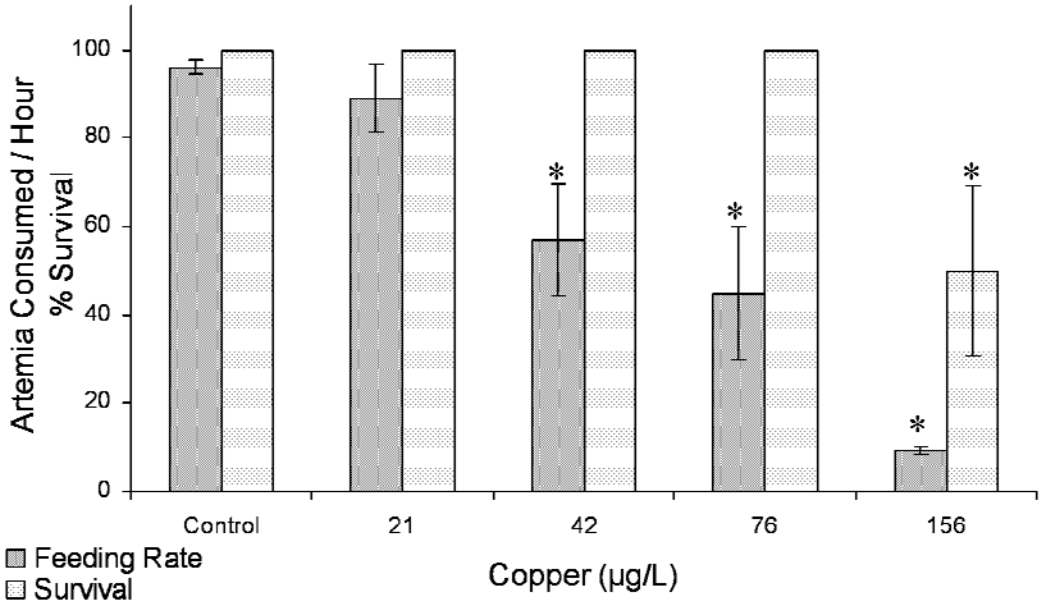

Figure 7-1. Survival and post exposure feeding rate results following 48 h copper exposure to N. arenaceodentata. Copper levels are average of start and end copper concentration. * denotes significance ($p<0.05$) from control.

Table 7-2. Comparison of the sensitivity of N. arenaceodentata between mortality and post exposure feeding rate endpoints. Dash indicates test solutions were not measured.

Compound	Endpoint	Nominal/ Measured	NOEC	LOEC	LC50/EC50 (95% CL)
Copper	96 h Mortality	Nominal	54	90	80 (71-90)
		Measured	-	-	-
	48 h Mortality	Nominal	100	200	200
		Measured	76	156	156
	48 h Feeding Rate	Nominal	25	50	72 (27-138)
		Measured	21	42	57 (31-91)
Phenanthrene	96 h Mortality	Nominal	1000	2000	1180 (876-1617)
		Measured	-	-	-
	48 h Mortality	Nominal	1400	2400	4083
		Measured	669	1369	2224 (1499-5037)
	48 h Feeding Rate	Nominal	310	520	814 (145-1519)
		Measured	104	210	345 (62-689)

Table 7-3. Results of the chemical analysis of copper (µg/L) from 48h survival and post exposure feeding rate experiments. Solutions were pooled from replicates (N=4).

Nominal	Initial	Final	Average
Control	4.5	4.5	4.5
25	23.0	19.2	21.1
50	44.4	38.6	41.5
100	81.2	70.7	75.9
200	159	153	156

PAH experiments

The dose-response relationship for measured phenanthrene is shown in Figure 7-2. Toxicity metrics and chemical analysis of tests solutions are shown in Table 7-2 and Table 7-4, respectively. The 96 h phenanthrene exposure resulted in one mortality in the 1000 µg/L treatment and complete mortality in 2000 µg/L treatment. It was clear that the worms were under great stress, with discoloration, lack of tube formation and lack of clinging to the exposure vessels in 500 µg/L concentration.

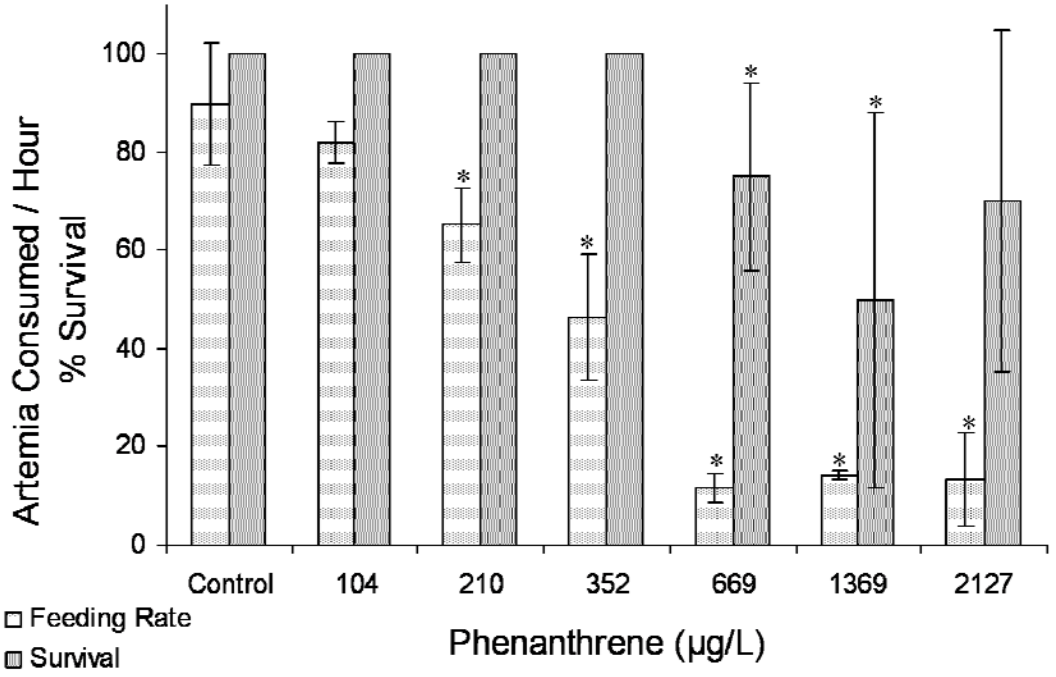

Figure 7-2. Survival and post exposure feeding rate results following 48 h phenanthrene exposure to N. arenaceodentata. Phenanthrene levels are average of initial and prior to renewal measured concentration. * denotes significance ($p<0.05$) from control.

Table 7-4. Results of the chemical analysis of phenanthrene solutions (µg/L) from 48 h survival and post exposure feeding rate experiments. The 24 h data represent concentrations immediately prior to the renewal of the exposure water.

Nominal	Initial	24 Hour	Average
310	207	0.68	104
520	385	34.3	210
860	567	137	352
1400	1011	327	669
2400	2139	598	1369
4000	3246	1007	2127

For the 48 h exposure, mortality was observed in the highest three treatments, but the highest concentration did not exhibit significant mortality (P=0.09; Figure 2). The feeding rate assay exhibited a very strong dose-response relationship with phenanthrene concentration (Figure 2). Statistically significant effects were observed as low as 210 ug/L, yet the highest three concentrations did not differ dramatically from one another. Based on the toxicity metrics, feeding rate was more sensitive than lethality by a factor of greater than six.

Chemical analysis revealed about a 70% reduction in phenanthrene for most concentrations (and a 92% reduction in the lowest exposure concentration) between the initial and 24 h exposure solutions prior to

renewal (Table 7-3). An average of the initial and 24 hour measured concentration was used to express the toxicity data.

Temperature and Salinity Effects

Of the variables tested, the only statistically significant reduction (relative to the normal conditioning combination; 20 °C/34 ‰) in feeding rate occurred in the 15 °C treatment (Figure 7-3). The feeding rate increased with temperature; at the control temperature (20 °C), the variability of the test increased with declining salinity, but feeding rate was not reduced significantly. At 25 °C, the feeding rates did not differ significantly from the 20 °C treatments at equivalent salinities. Significant reduction in the feeding rate was observed in all the 15 °C treatments when compared to 20 °C treatments.

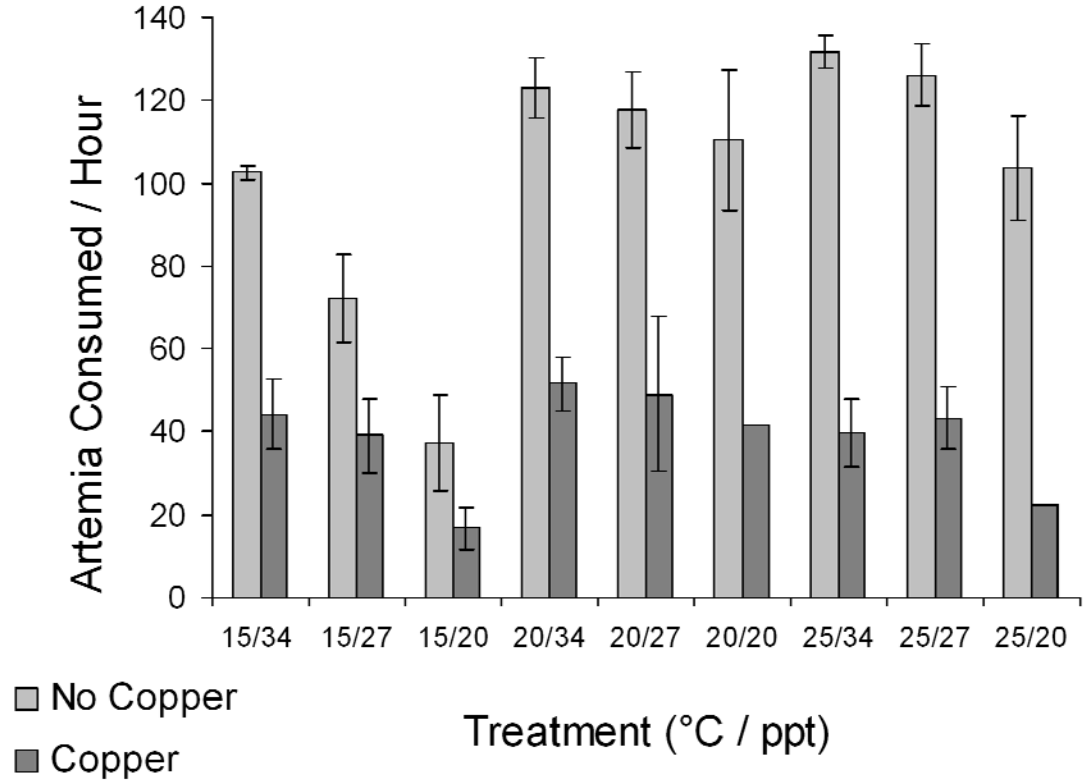

Figure 7-3. Post exposure feeding rate of N. arenaceodentata following 48 h exposure to various temperature and salinity combinations.

Decreasing salinity had some effect on feeding rate but was only significant at 15 °C, where feeding rate was already significantly decreased by temperature. These trends were mimicked in the copper-spiked samples with a slight dose dependent trend evident at 15 and 20°C treatments. The concentration of copper (nominal 80 µg/L) used approximated the EC50 determined previously and produced a corresponding reduction in feeding rates (Figure 7-3). The most depressed feeding rates in the spiked samples occurred in the same treatments as the unspiked samples.

7.5. Discussion

Comparability between two species in copper sensitivity

This study showed that post exposure feeding rate appears to be a very promising endpoint for marine and estuarine toxicity exposures with *N. arenaceodentata.* The presented post exposure feeding rate assay with *N. arenaceodentata* is based on similar work by Moreira et al (2005), with some alterations due to differences between the two species of polychaete.

H. diversicolor is a related Nereidae family polychaete and shares many of the same ecological roles as *N. arenaceodentata* (Scaps, 2002). *H. diversicolor* is resistant to variable environmental conditions as they inhabit estuarine habitats, while *N. arenaceodentata* is predominantly found in intertidal and subtidal environments (Pesch and Schauer, 1988) and less tolerant to low salinities (ASTM 1611). The feeding structure and habits of both species are similar enough that both species will eat whole *Artemia franciscana* nauplii. Feeding rates were also similar, with *N. arenaceodentata* capable of consuming more nauplii (up to 100 nauplii/hour) in the feeding phase than *H. diversicolor* (~70 nauplii/ hour; Moreira et al. 2005) under typical exposure conditions (34 ‰, 20 ºC) for the former.

Both species are benthic omnivores that form irrigated burrows, but *H. diversicolor* is much larger (adults being approximately 14 mg vs. 2 mg dry weight for *N. arenaceodentata*), and does not build a mucous lined tube. The tube building of *N. arenaceodentata* could potentially reduce the exposure of this species to sediment-bound contaminants, with most of the exposure coming from the overlying water (Dillon et al, 1993) or from ingestion of organisms in the water column or on the sediment surface.

Although there are distinct differences between the two species, laboratory copper exposures resulted in similar effects. The 48 h LC50 of 156 µg/L for *N. arenaceodenata* is comparable to the reported 48 h LC50 of 125 µg/L for *H. diversicolor*, while the 48 h EC50 (based on post exposure feeding rate of 57 µg/L for *N. arenaceodentata* is also comparable to the 48 h EC50 of 52 µg/L for *H. diversicolor*. For both species, the feeding rate endpoint was approximately three times more sensitive than lethality.

Phenanthrene effects

Feeding essentially ceased in the three treatments with the highest phenanthrene concentration. Significant ($p<0.05$) mortality was observed at 669 and 1369 µg/L, but was more variable in the highest exposure concentrations (2127 µg/L). Surviving organisms in that treatment, however, were discolored, did not cling to the exposure vessels, and did not form mucous tubes, as was observed in their lower dose counterparts. These animals were likely to have died with continued exposure, but were still considered alive as they were still motile. The reported solubility of phenanthrene in seawater of 600 µg/L (Rossi and Neff, 1978), suggests that phenanthrene would not be completely dissolved at the higher concentrations (669-2127 µg/L), thus potentially reducing exposure and explaining the overall similarity in responses, for both mortality and feeding rate, at those concentrations.

Chemical analysis of phenanthrene exposure solutions indicated a substantial reduction in phenanthrene levels in all treatments. Table 7-4 shows that the initial level of phenanthrene was relatively close to the nominal levels. The subsequent decline in phenanthrene levels for all treatments, however, was likely the result of volatilization and possibly photo-oxidation (Rossi and Neff, 1978). Phenanthrene metabolism by cytochrome P450 enzyme might also act to reduce concentration in solution, although the extent to which *N. arenaceodentata* and related polychaetes can uptake and transform phenanthrene is not completely understood (Jørgensen et al 2008).

The most likely mechanism of toxicity seen in exposure to phenanthrene is narcosis (Landrum et al. 2003). In their studies of phenanthrene effects on emergent juvenile *N. arenaceodentata*, Emery and Dillon (1996) speculated that reversible inactivity following exposure to sub-lethal phenanthrene concentrations can affect growth via feeding inhibition. This feeding inhibition can be directly observed

with increasing phenanthrene concentration in our experiments, providing additional relevance for the feeding rate endpoint.

Effects of temperature and salinity

Because *in situ* toxicity testing is subject to reduced control of certain environmental variable, understanding how the test method is affected by such conditions is crucial to explaining potential variability observed in field deployments. In this study, feeding rate was somewhat dependent on the environmental conditions of the test. While reduced salinity did negatively affect the feeding rate, lower temperature had a much greater impact. Although greater than standard temperatures did seem to increase feeding rate, any changes were not statistically significant (Figure 7-3). Due to the synergistic stress, feeding rates were much more reduced when both temperature and salinity were lower than standard test conditions. It should be noted, however, that the temperatures and salinities selected for this experiment were by no means the extremes, but rather conditions that likely bracket what environments this assay might be relevant for, based on previously published threshold data for these parameters (Dillon et al. 1993). It is also interesting to note that the sub-lethal copper dose resulted in essentially the same feeding rate trends as without copper, aside from the overall reduction in feeding rate that was expected at this copper concentration under all treatment combinations. The same trends with and without the metal spike suggests that, at least for copper, fluctuations in salinity and temperature in the field should not confound interpretation of data that is collected from field deployments where these parameters vary to some degree.

The positive correlation between feeding rate and temperature isn't surprising considering that most poikilothermic organisms tend to have reduced metabolism at lower temperature. The positive relationship between feeding rate and salinity, which was most clearly apparent at the lowest test temperature, could be related to physiological stress experienced by the polychaetes at the lower temperatures. *N. arenaceodentata* is typically found in relatively saline environments, is typically cultured at a salinity of 34 ‰, and did not survive long term laboratory exposure at salinities less than 20 ‰ (Dillon et al. 1993).

Potential uses of this assay

The feeding rate assay with *N. arenaceodentata* has several potential advantages for site assessment. Test organisms can be deployed in the field for exposure while the assay takes place in the laboratory giving researchers more control in the quantification phase of testing. The sensitivity of the feeding rate to subtle sub-lethal effects provides an opportunity to rapidly identify sites that might not show an acute lethal response without the necessity of performing growth or reproduction studies, which are substantially more complex for *in situ* assessment in particular. An *in situ* exposure period imparts the ability to accurately assess actual site toxicity without potential confounding factors such as sample retrieval, storage and processing.

Potential confounding factors associated with this assay with respect to use in the field, however, should be noted, and include organism handling during various phases of the protocol, possible presence of infaunal *N. arenaceodentata*, *in situ* food variability, and environmental (non-contaminant) stressors such as variation in temperature, salinity, dissolved oxygen, and presence of predators/competitors in sediment samples. Appropriate measures should be taken to minimize these confounding factors and/or improve data interpretation for *in situ* exposures with this endpoint, including the use of travel controls (Anderson et al. 1998, Burton et al. 1996) to compare with laboratory controls, and continuous monitoring of environmental conditions at the test site using commercially available monitoring instruments.

Indigenous animals present a serious challenge, as their unintentional inclusion in the feeding assay might significantly differ from worms deployed in the field as the native *N. arenaceodentata* will most likely have been acclimated to site-specific toxicants. Pesch and Hoffman (1986), for instance, have shown the *N. arenaceodentata* have the ability to acclimate to long term exposures of low levels of copper. If the

presence of infaunal species that are not easily distinguishable from the test organisms, the use of a non-toxic marking on the test animals would prove useful in indicating which organisms to use in the feeding rate portion of the assay. Crane et al (2000) report using this method for marking amphipods prior to deployment.

Potential for use in field studies

Use of *N. arenaceodentata* in *in situ* post exposure feeding rate assays as a rapid toxicity assessment tool shows promise based on the presented laboratory experiments. The authors are currently investigating the use of this assay in short-term field exposures at a variety of sediment sites, and have thus far observed high recoveries and feeding rates that correlate with other concurrently deployed endpoints as well as with historic sediment contamination (unpublished data).

Most short-term bioassays are sensitive only to high concentrations of contaminants (Greenstein et al, 2008), so a short-term sensitive bioassay such as the post exposure feeding rate assay with *N. arenaceodentata* potentially fills a necessary gap in the currently available methodology. Relative to strict laboratory exposures, the assay increases ecological relevance and potential for more accurate assessment of effects by potentially conducting exposure in the field, yet provides traditional laboratory levels of control by assessing the sublethal endpoint under more controlled conditions.

7.6. Acknowledgements

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8.0 DETAILED SEA RING HARDWARE DESCRIPTION

8.1. Introduction

In order to meet the requirements for conducting a variety of synoptically collected *in situ* measurements in deep water marine and estuarine environments, a diver deployed modular platform was developed. The prototype Sediment Ecotoxicity Assessment Ring (SEA-Ring; Figure 8-1, Figure 8-2) consists of a circular carousel capable of housing an array of in-situ toxicity and bioaccumulation chambers, passive sampling devices, and water quality sensing devices (Figure 8-1, Figure 8-2). The SEA-Ring can be used to assess potential impacts due to a variety of contaminants and exposure pathways, and has the advantage of improving the overall linkage between exposure and effects when compared to traditional laboratory test approaches alone (e.g. grab sample testing under artificial conditions).

8.2. Main platform

The carousel was made from ½" acrylic. The base and top of the carousel were circular in shape with diameters of 17 and 13", respectively (Figure 8-1). The base portion possessed 14 circular cutouts, each 3 1/8" in diameter. A 5 ½"-long cylindrical chamber holder (1/8" acrylic) was glued (Weld-On #16 clear acrylic solvent cement) into each cutout, and served as a means of housing the individual exposure chambers. Each chamber holder possessed 12 vertically oriented cutouts approximately 3" long by ½" wide so as to maximize water flow across the mesh covered exposure chambers while maintaining structural rigidity of the holder.

8.3. Exposure chambers

Three basic types of exposure chambers were designed, those for conducting water column (WC), sediment-water interface (SWI), or surficial sediment (SED) exposures (Figure 8-3). The WC and SWI exposure chambers were 5" long, while the SED chambers were 10" long. Exposure chambers were designed using various characteristics of those successfully demonstrated by others (i.e. Anderson et al. 2004, Burton et al. 2005). The 5" chambers were used for WC or SWI exposures. These chambers were maintained above the sediment surface with acrylic stops that were glued onto the bottom inside lip of the chamber holders, while the SED chambers extended approximately 5" below the base portion of the SEA Ring in the sediment. Exposure chambers were made of cellulose acetate butyrate cylindrical tubing (CAB tubing, #KM-2340, k-mac-plastics.net), and were 1/16" thick with an inner diameter of 2 5/8" (2 ¾" outer diameter). Chambers used for housing smaller organisms such as amphipods, polychaetes, mysids, or bivalve embryos each possessed two mesh cutouts, approximately 2 ¾" tall by 1 ¾" wide. Mesh pore size was typically 250-500 µm, except for bivalve embryos (11 or 20 µm mesh). Mesh was fastened to the cutouts with aquarium grade silicone glue (DAP Clear Aquarium Sealant 100% Silicone). Exposure chambers housing larger organisms (i.e. adult bivalves) typically utilized chambers to which 1/8” holes were drilled approximately every ¾” around and down the tube to maximize water flow, and did not require mesh.

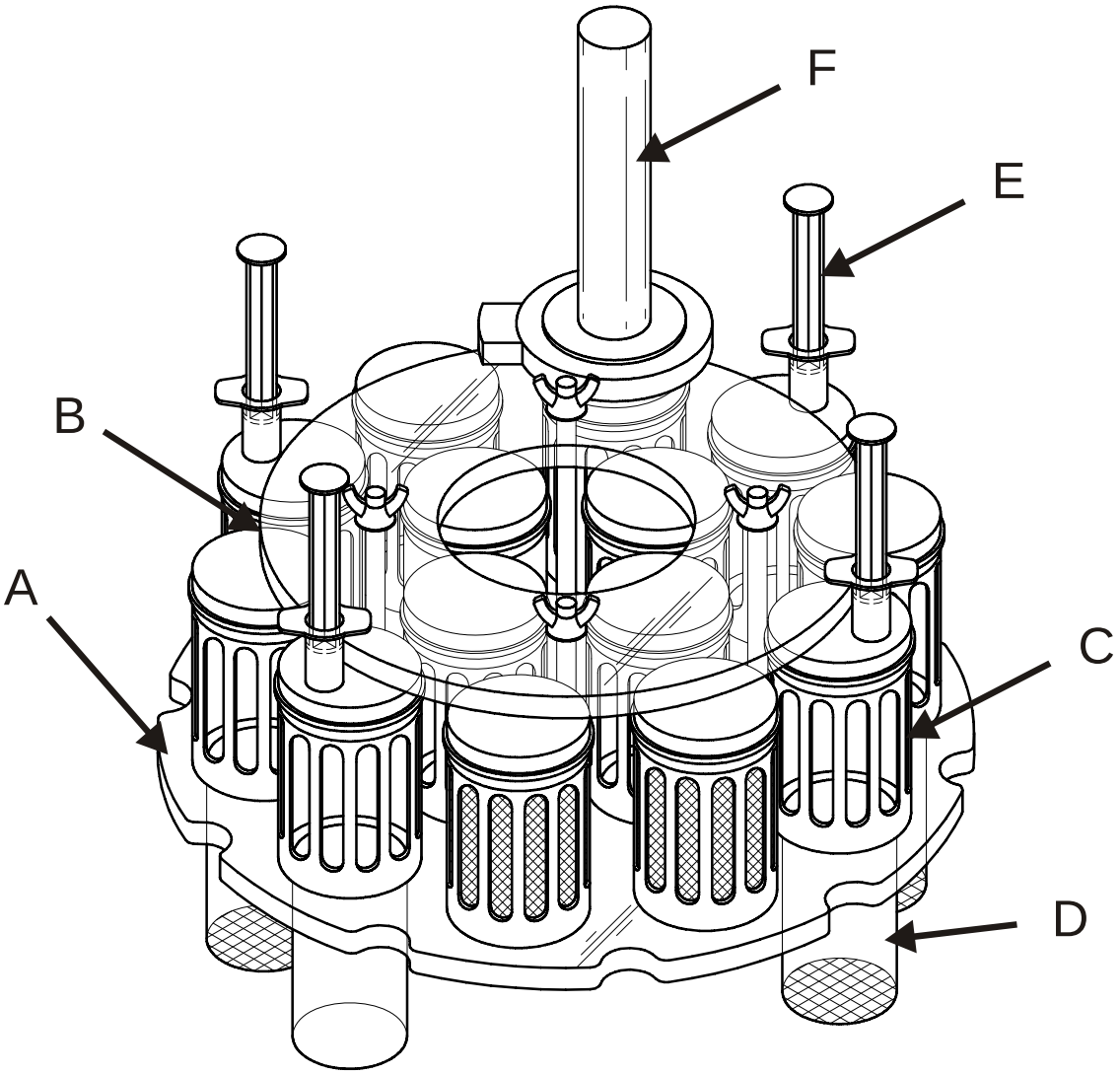

Figure 8-1. Drawing of the Sediment Ecotoxicity Assessment Rings (SEA Ring) used at the test sites. A) base plate; B) top plate; C) chamber holder; D) exposure chamber; E) syringe for dispensing sediment dwelling organisms; F) in situ water quality sensor.

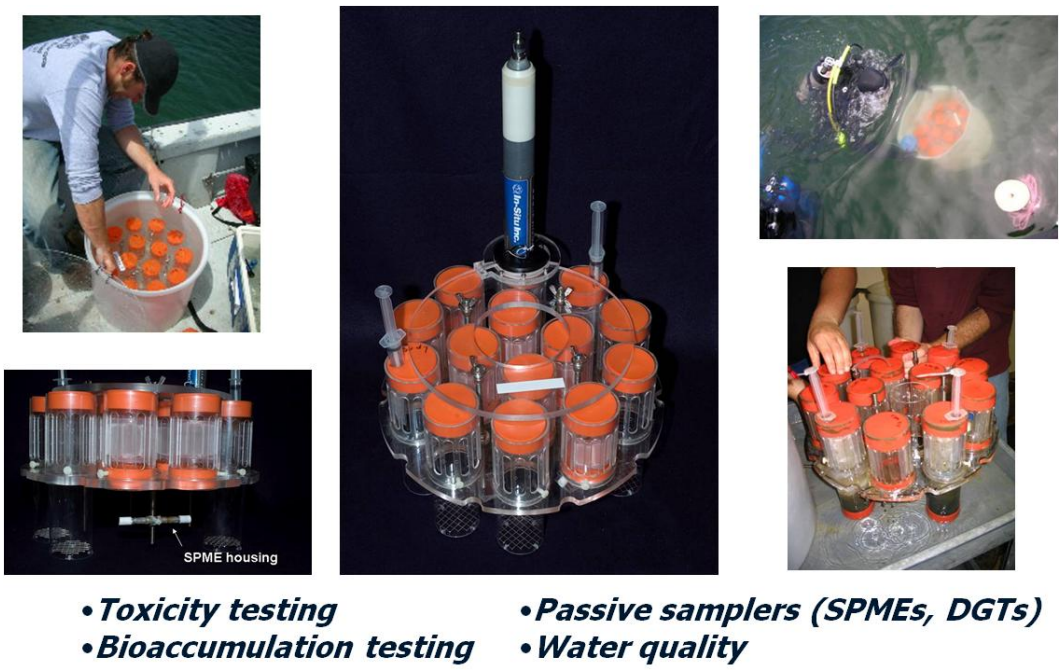

Figure 8-2. Photographs of SEA Ring test system.

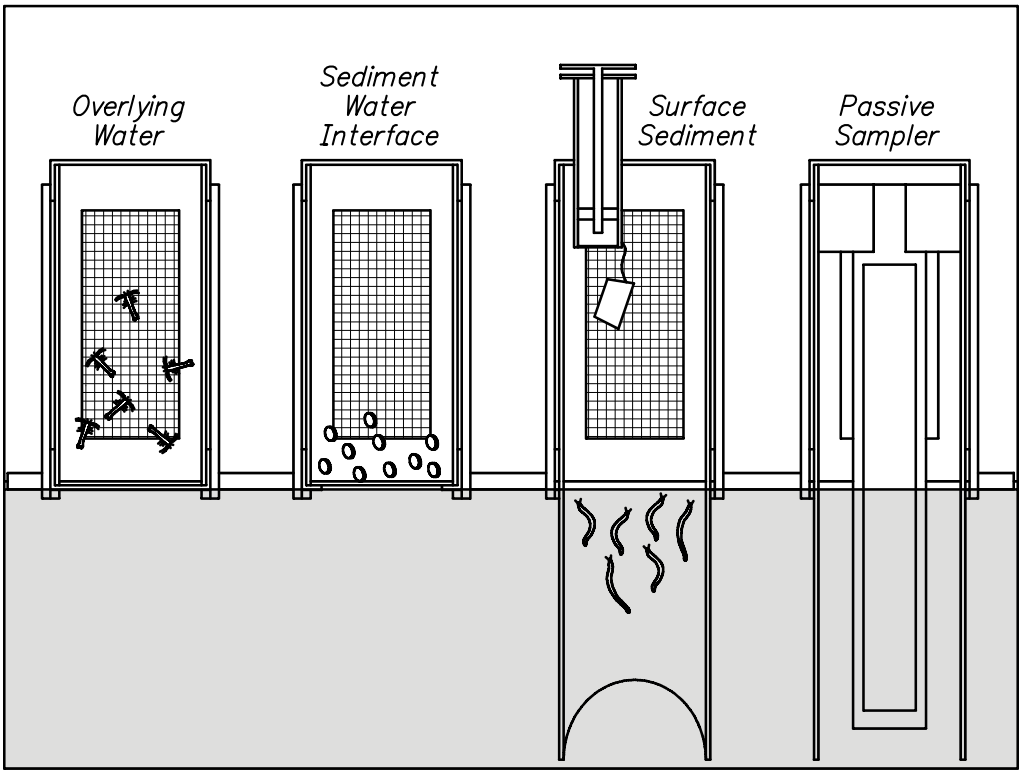

Figure 8-3. Side view of exposure chambers, including options for overlying water, sediment-water interface, or surface sediment exposures. Passive samplers are also integrated into chambers, as shown for DGT.

8.4. Exposure chamber closure

Exposure chamber contents were enclosed using polyethylene end caps (www.caplugs.com, 2 ¾" diameter, Niagara, Item #2321AH1). The WC chambers generally possessed one unadultered cap on the top and bottom. The bottom cap on SWI chambers was modified by placing a circular mesh covered acrylic ring inside an end cap to which a 2 ½" circular cutout was made to allow for exposure to potential contaminant flux at the sediment-water interface. The interface exposure concept was based on designs used for toxicity testing at the SWI in the laboratory (Anderson et al. 1996). The longer SED chambers were either open at the bottom (for smaller organisms) or held into place with nylon screws built into the base of the chamber holders (Figure 8-1, Figure 8-3, Figure 8-3). The SED chambers housing smaller organisms were open at the bottom, and capped from the bottom prior to recovery.

8.5. Organism delivery to sediment chambers

Organisms used in WC and SWI exposures were typically loaded directly into the relevant chambers in the laboratory. This step was conducted in circular 17 gallon HDPE chambers (Chem-Tainer, Part #TC1815AA/AB), which were also used for transportation of the SEA Rings to the field site. Smaller organisms used in SED exposures were loaded into modified 20 or 30 mL plastics syringes, similar to those used by Anderson et al. (2004). The luer-lock (bottom) portion of the syringe was removed and silicone stoppers (size #3) retained the organisms in clean seawater until deployment. The loaded syringes were transported to the test site in coolers filled with seawater at the test site temperature and salinity. Just prior to deployment, the syringes were inserted into previously drilled holes through the top end caps. Once the SEA Rings were positioned on the sea floor, organisms were released by manually depressing the syringe plunger, or remotely from the water surface, thus releasing the silicone stopper.

8.6. Integrated water circulation

Initial deployments, particularly those with small mesh and/or extended exposure duration, indicated that screens fouled and reduced water flow and water quality. Subsequent exposures (i.e. NAS Pensacola, Chollas Creek) included an integrated water circulation system. A small (2 watt) submersible pump was attached to a custom-built waterproof battery housing containing three lithium D cell batteries (Xeno XL-2OSF, 3.6V). Mesh with a pore size of 250 µm was attached to the pump intake to reduce clogging, and 3/8” I.D. Tygon tubing gently sprayed site water through small holes strategically placed adjacent to exposure chamber windows on the interior of the SEA Ring.

8.7. Water quality sensing

Water quality was measured continuously inside representative exposure chambers using a portable Troll 9500 (In Situ, Inc.) multi-parameter water quality monitoring and logging instrument. The top cap of a typical exposure chamber was modified so that the sensors were fully enclosed (Figure 8-1). This allowed for continuous monitoring of various water quality parameters (i.e. pH, temperature, salinity, dissolved oxygen, ORP, and depth) not only on the sea floor, but specifically inside a chamber that represented conditions encountered by the test organisms. Discrete water samples were also collected on a subset of exposure chambers through ¼" tubing (which contained mesh on the bottom portion). Discrete water sampling was conducted with a 40 mL plastic syringe that attached to the tubing, and sample measured immediately by field crew.

8.8. Deployment approach

SEA Rings were loaded in the laboratory and transported to the field site by boat in HDPE storage containers (ChemTainer Industries, Inc.) filled with clean seawater of the approximate site temperature and salinity. Once on site, the storage containers were gently lowered into the water, and the SEA Rings removed by divers, who placed them on the sea floor. If SED exposure chambers were used, syringe stoppers were popped by pushing down on the plungers, allowing organisms to fall towards, and burrow into, the sediments. The SED chambers served as anchors, preventing loss of the device due to site

conditions. Passive samplers were deployed in modified SED chambers (Figure 8-3), attached at a fixed depth on holding devices on the SEA Ring frame, or along the perimeter of the SEA Ring.

A set of travel controls accompanied the deployed organisms to the field site. Travel controls were housed either in syringes or WC/SWI chambers in ice chests containing seawater of the approximate site temperature and salinity, and brought back to the laboratory for observation.

8.9. Recovery approach

After the specified exposure duration, divers retrieved SEA Rings by first assessing the overall condition of the exposure chambers and removing overlying water samples through small ports (1/4" tubing with mesh on the inside) installed into select end caps. These water samples were immediately assessed by the boat crew for water quality (i.e. pH, temperature, salinity, DO, ORP). The open (bottom) end of SED chambers were then capped by gently covering with PE end caps with the SEA Ring still in place. At Chollas Creek, an alternate approach for capturing sediment from SED chambers was employed (see Refinements section below). The SEA Rings were subsequently lifted off the sea floor, placed into the appropriate ChemTainer, and brought back up to the water surface. Once on the boat, initial observations were made as to integrity of the exposure chambers (i.e. mesh screens). SEA Rings were then transferred back to the laboratory for processing.

8.10. Refinements

Several refinements to the prototype SEA Rings were made following lessons learned from each of the test site deployments. This included incorporation of the recirculating water pump system (discussed above) as well as several modifications to improve reliability as well as reduce dependence on diver support. Reduced diver dependence was achieved by incorporating a bracket to the top portion of the SEA Ring to which a series of poles could be inserted for deployment from a boat. Once adequately positioned on the sediment surface, a pin attached by a line was pulled allowing the pole to be pushed to a second tier, which triggered release of SED test organisms housed in modified plastic syringes. Recovery of open bottomed SED chambers was addressed by modifying the polyethylene end caps with a series of cross-sectional slices (kept open with an acrylic ring during deployment) that would serve much like a core catcher, trapping sediment and test organisms when pulled from the sediment.

9.0 AN INTEGRATED EXPOSURE AND EFFECTS ASSESSMENT APPROACH INVOLVING IN SITU AND LABORATORY TOOLS ALONG THREE CONTAMINATION GRADIENTS

9.1. Overview

This section describes the deployments at three field sites to demonstrate the SEAP approach (without the GIS Weight-of-Evidence analysis, see Section 10). A comprehensive, weight-of-evidence based approach integrating laboratory and *in situ* toxicity and bioaccumulation testing, passive sampler devices as biomimetics of exposure, hydrological characterization tools, continuous water quality sensing, and multi-phase chemical analyses was developed for potential use at contaminated sediment sites. The overarching goal of the approach is to improve the accuracy of the assessment of ecological risk and recovery at contaminated sediment sites by improving the linkage between exposure and effects, particularly where traditional laboratory-based methods alone are inadequate to make informed management decisions. The approach was designed to be conducted rapidly and cost effectively, with on-site assessments generally requiring less than one week.

Three sites were selected for development of the specific tools and overall approach, and included the pier areas at Naval Base San Diego (NBSD), an estuarine wetland at Naval Air Station Pensacola, and the mouth of Chollas Creek, a tributary of San Diego Bay. Tools employed at each site were selected based on historical knowledge of the contaminants of concern and expected exposure pathways, as well as with the concurrent assistance from ground water discharge characterization tools (i.e. Trident Probe, UltraSeep). All sites incorporated the SEA Rings (see previous chapter) as the platform for housing the various *in situ* devices.

Overall, the integration of various endpoints and measures was useful in characterizing the sites investigated. Toxicity, bioaccumulation`, bulk chemistry, and bioavailability based on pore water concentrations derived from uptake by passive sampling devices (PSDs) followed the expected gradient at NBSD, and suggested hydrophobic organic compounds (i.e. PAHs) were important stressors, while bulk metals and DGT concentrations appeared to be of less concern. At NAS Pensacola, similar results were observed. However, the Trident and UltraSeep were used to evaluate the potential for groundwater-surface water interactions to be contributing to historically defined effects at the southern end of the water body. Although groundwater was discharging into the surficial sediments, analysis of flow-weighted samples of the discharge revealed little to no chemical contamination associated with the infiltrating groundwater. Bulk chemistry, toxicity, and bioaccumulation, however, pointed to possible PAH toxicity at one station, which could have been exacerbated by UV photoactivation, explaining the difference between *in situ* exposure at the shallow station (<1 m) and laboratory toxicity results. The importance of continuous water quality sensing was very clear at the Chollas Creek site, where diurnal dips in dissolved oxygen may have contributed to amphipod toxicity. That site, however, appears to be improving based on lower bulk chemical concentrations and toxicity than previously observed. This could be associated with recent restoration efforts upstream and reduced inputs of organophosphate pesticides, but the potential for temporal and spatial variability of results was noted.

In general, the development and use of *in situ* bioassays at deep water marine and estuarine sites was successful, with water quality sensing assisting with the interpretation of results. Amphipod (*Eohaustorius estuarius* and *Leptocheirus plumulosus*), polychaetes (*Neanthes arenaceodentata*), and mysids (*Americamysis bahia*) were the most robust yet responsive organisms employed as *in situ* test species. While survival or bioaccumulation served as the endpoint for most species, post exposure feeding rate (see Section 7.0) with *N. arenaceodentata* appears to be a responsive and practical sub-lethal endpoint ideal for short-term (i.e. 48 hour) *in situ* assessments. Mussel (*Mytilus galloprovincialis*) embryogenesis exposures at the sediment-water interface, however, were complicated by the agglomeration of very fine particles that passed through the mesh, making microscopic examination not

practical, if not impossible. New designs and applications for *in situ* use of this endpoint continue to be investigated.

Field-deployed passive sampling devices were useful for characterizing labile metals (diffusive gradient in thin films; DGT), while PDMS solid phase microextraction fibers were generally positively correlated with uptake by small benthic invertebrates, providing additional evidence of the utility of these tools as biomimetics. This is a significant result as sediment quality benchmarks (e.g. Long et al. 1995) are often based on the solid-phase concentrations, which appear to be a weaker indicator of bioaccumulation potential than pore water concentrations.

In addition to utility at sites where exposure is ephemeral in nature (i.e. groundwater-surface water interaction, storm water discharges, oil spills), it is anticipated that the *in situ* approach will be valuable towards the assessment of effectiveness of in-place sediment management strategies, which might otherwise present challenges using traditional laboratory approaches.

9.2. Introduction

Characterization of ecological risk or recovery in aquatic systems has traditionally been assessed by collection of water or sediment samples from potentially contaminated sites followed by conducting laboratory toxicity and bioaccumulation tests, comparing water or sediment chemical concentrations to various benchmarks, and/or conducting surveys of benthic macroinvertebrate community structure (Chapman et al. 1990). This approach, though well-established and accepted by environmental regulators, may not accurately assess ecological risk for a variety of reasons.

In situ assessment approaches potentially provide better linkages among chemical exposure, biological uptake, and response by providing more realistic exposures that 1) reduce sample manipulation, thus preserving natural chemistry gradients and bioavailability; 2) integrate both natural (i.e. tide, currents, temperature, pH, light, sediment disturbance) and anthropogenic stressors; 3) include exposure to volatile or time varying stressors lost in grab sampling (i.e. groundwater-surface water interaction, storm water discharges, oil spills); and 4) provide options for exposure source identification by compartmentalizing the exposure (Burton et al. 1996; Chappie and Burton 1997; Phillips et al 2004; Liber et al. 2007; Rosen et al. 2009). For these and other reasons identified by those authors, it is frequently recommended that *in situ* bioassays be part of a weight-of evidence approach in the risk assessment process (e.g., Wharfe et al. 2007, and references therein).

In addition, sediment quality guidelines (SQGs; e.g., Long et al., 1995; MacDonald et al., 1996) frequently used to assess the potential for aquatic life impacts, are typically based on bulk-phase chemical concentrations, which don't take contaminant bioavailability into account. Contaminant uptake by organisms is one way for interpreting bioavailability, but passive sampling devices (PSDs) are increasingly being developed and demonstrated for their ability to provide a cost effective, simpler, and potentially less variable means of assessing pore water concentrations that better represent bioavailability than bulk phase concentrations.

The purpose of this research was to develop an efficient, accurate and integrated approach for the assessment of ecosystem risk and recovery at sites where contaminated sediments exist, or previously existed. The study involved the development of an integrated exposure and effects assessment approach involving synoptic, rapid *in situ* hydrological, chemical, biological, and toxicological measurements for providing concise, decision-oriented scientific and ecological information to improve the overall management of contaminated sediment sites. A unique ability to simultaneously assess these interdependent processes was achieved by integrating multiple tools/assays simultaneously to better link exposure and effects measures. This included placing *in situ* toxicity and bioaccumulation tests, passive sampling devices (PSD) as potential surrogates for biological uptake of both metal and hydrophobic

organic contaminants (HOC), tools for groundwater discharge characterization in surficial sediments, and continuous water quality sensing in an integrated approach and integrated exposure systems.

9.3. Materials and Methods

9.3.1. Approach

This integrated approach involved concurrent laboratory and *in situ* toxicity and bioaccumulation exposures, passive sampler devices (PSDs) for assessment of bioavailable metals and organics, sediment and/or pore water chemistry, continuous water quality monitoring, and in some cases, groundwater discharge characterization assessment. The *in situ* toxicity testing tools used were identified and optimized in Task 1 of this project, published in a SPAWAR Technical Report (Rosen et al. 2009), and frequently conducted alongside more traditional laboratory exposures (i.e. toxicity tests) to help make a more accurate assessment of ecological risk.

The *in situ* exposures were conducted using the Sediment Ecotoxicity Assessment Ring (SEA Ring) design described in detail in Section 7.0. Briefly, SEA Rings served as platforms for housing multiple *in situ* exposure chambers. To identify exposure source, chambers were uniquely designed and concomitantly deployed to assess exposure from surficial sediment (SED), the sediment-water interface (SWI), or the water column (WC). In general, organisms naturally inhabiting a particular compartment were selected for exposure (i.e. mysid shrimp were generally used in WC or SWI exposures and burrowing amphipods were generally used in SED exposures). Typically three SEA Rings, each containing up to 14 exposure chambers, were deployed at each station. A minimum of four replicate chambers were used for each test endpoint. Amphipod chambers typically housed 20 organisms each, while polychaete, mysid, and infaunal bivalve chambers were typically loaded with 10 organisms each. Mesh screen (Nitex) sizes were generally 250-500 µm for larger organisms (e.g. amphipods, polychaetes, mysids), and 11-20 µm for smaller (microscopic) organisms (e.g. mussel embryos). Water exchange in chambers housing larger organisms (e.g. for infaunal bivalve bioaccumulation exposures) was typically provided by a series of 3/16" diameter holes drilled through the chambers instead of mesh screens. Specific design details and discussion on loading of chambers, transfer to the test site, chamber water circulation system, and other details pertaining to the SEA Ring design and use are provided in Section 8.0.

Water quality parameters (i.e. pH, temperature, dissolved oxygen, salinity, depth, oxidation-reduction potential) were continuously measured using a Troll 9500 (In Situ, Inc.) water parameter sensor. One Troll was positioned at each station. Rather than monitoring external parameters, water quality was measured inside a representative SED exposure chamber (electrodes positioned just above the SWI), for best interpretation of the organism exposure results.

For two of the sites, all phases of preparation and breakdown occurred at the SSC Pacific Bioassay Laboratory (San Diego, CA), while the NAS Pensacola study was staged out of the toxicology laboratory at EPA Gulf Ecology Division, in Gulf Breeze, FL, within just a few miles from the study site.

9.3.2. Test organisms

A number of different species representing different phyla, feeding habits, exposure routes, and endpoint sensitivities were utilized for toxicity and bioaccumulation exposures. Test organism selection was also based on relevance to, or presence at, the test location as well as what was used in historical or concurrent laboratory studies.

All organisms were provided by commercial suppliers, and were either field-collected or cultured. The marine amphipod *Eohaustorius estuarius* (3-5 mm) was collected from Yaquina Bay, OR (Northwestern Aquatic Sciences, Newport, OR), while the amphipod *Leptocheirus plumulosus* (3-5 mm) and mysid shrimp *Americamysis bahia* (3-5 days old) were cultured by Aquatic Biosystems (Fort Collins, CO). The

polychaete *Neanthes arenaceodentata* (6 week old) was cultured by California State University Long Beach, CA. Adult mussels were provided by Carlsbad Aquafarms (Carlsbad, CA). *Mercenaria mercenaria* (2 cm) were provided by Southern Cross Sea Farms (Cedar Key, FL). *Musculista senhousia* (2 cm) were collected from a reference site on the west side of San Diego Bay, CA.

All test organisms were shipped overnight to the relevant laboratory and acclimated in uncontaminated seawater adjusted to the approximate site conditions for 24-48 h prior to deployment.

9.3.3. Controls and reference sites

Laboratory toxicity tests included control sediment, which consisted of the home sediment for *E. estuarius* (Yaquina Bay, OR) and clean sediment from Sequim Bay, WA for *L. plumulosus.* Laboratory control/dilution water was 0.45 µm filtered seawater from the research pier at Scripps Institute of Oceanography (La Jolla, CA), or comparably filtered water pumped into the laboratory at SSC Pacific from near the mouth of San Diego Bay, for San Diego based studies. The control/dilution water for the Pensacola-based study was 0.45 µm filtered seawater from Santa Rosa Sound (Pensacola, FL).

In situ exposures typically included both a laboratory and a travel control. Travel controls were used to assess response from stress associated with transportation to and from the field (Anderson et al 1998). Travel controls were treated the same as *in situ* deployed organisms in that they were caged and transported to the site, but were not deployed, and observed for the duration of the exposure in the laboratory. Comparisons were made to the laboratory control unless travel controls were substantially different in response from the laboratory controls.

9.3.4. Toxicity and Bioaccumulation Exposures

Toxicity and bioaccumulation exposures were generally based on standard laboratory protocols (i.e. USEPA 1994, USEPA 1995, USEPA/USACE 1998). However, exposures were frequently limited to 48 to 96 h exposures, which in some cases were shorter in duration than the standard methods (e.g. amphipod toxicity and bivalve bioaccumulation exposures). The intent was not necessarily to achieve steady state body residues, but rather develop a rapid assessment approach that could be integrated with other assessment tools that are frequently used over short time periods (e.g. a few tidal cycles). There is no readily apparent reason, however, that the tools developed in this study could not be used in exposures of longer duration.

Endpoints included amphipod survival and bioaccumulation, mysid survival, bivalve embryo-larval development, infaunal bivalve bioaccumulation, and polychaete post-exposure feeding rate. The feeding rate assay using *Neanthes arenaceodentata* was developed as part of SERDP #ER-1550 to develop a relevant short-term endpoint that would be indicative of sublethal effects, and is described in detail in Section 7.0 (Miller and Rosen, submitted). The endpoint is individual worm consumption rate of *Artemia* sp. nauplii.

Toxicity assessment was conducted immediately upon return to the laboratory (within 30 minutes of retrieval). Survivors were enumerated immediately upon recovery from chamber or sieves, bivalve larvae were preserved in buffered formalin for microscopic examination, and post exposure feeding rate assessment was initiated following a 1 h acclimation period to laboratory conditions in clean seawater.

Bioaccumulation organisms were purged for 4-24 h in clean seawater, to prevent excessive elimination or transformation of lighter weight contaminants, and frozen for extraction and analysis. Tissue analysis was conducted using a micro-extraction technique for use with small masses (Jones et al. 2006), and was conducted at the USACE Engineer Research and Development Center (Vicksburg, MS, USA).

9.3.5. Passive Samplers- SPME

Passive sampler devices are abiotic devices that can be suspended in water, porewater, or in sediments and act as a sink to which metals or hydrophobic molecules can partition. They have been widely used in recent years for sampling metals and organics in aquatic systems and found to be good indicators of fish and invertebrate bioaccumulation (i.e., biomimetic) (e.g., Arthur and Pawliszyn 1990; Huckins et al. 1993; Wells and Lanno 2001).

Solid phase microextraction fibers (SPMEs) are efficient and simple for monitoring of organic chemicals. Among other SPME materials, polydimethyl siloxane (PDMS) coated glass fibers show promise as a biomimetic for assessing sediment quality (Mayer et al. 2000), most of which has been demonstrated in the laboratory (e.g. You et al. 2006; Trimble et al. 2008). To demonstrate the ability of the PDMS pore water sampling method to predict *in situ* bioaccumulation potential of PAHs in marine systems, they were deployed in tandem with the SEA Rings (positioned around perimeter) at each of the three study sites within close (~1-2 inches) proximity to the bioaccumulation exposure chambers. Upon retrieval (2-21 days, depending on site), the PDMS fibers were immediately cleaned, processed into solvent in 5-cm intervals, and analyzed for PAHs. Organisms from the cages were separated from the sediment and allowed to depurate for 24 hours before tissue and lipid extractions. This work was conducted in collaboration with the University of Texas, Austin under ESTCP Project #ER-0624 (Demonstration and Evaluation of Solid Phase Microextraction for the Assessment of Bioavailability and Contaminant Mobility). The approach is described in detail by Lampert et al. (in prep), with some brief points made below.

Most SPME development has taken place in laboratory studies. To conduct this work in the field, an *in situ* apparatus for deploying the PDMS fibers was developed. To protect the fibers in the sediment column, a stainless steel piezometer was used as a tool to insert and recover the PDMS fibers into the sediment environment. An approximately 2-mm wide rectangular groove was made in the inner rod of the piezometer to serve as a frame for the fragile PDMS fibers. Approximately 0.5-mm thick slits were cut into the outer part of the piezometer at ¼" spacing to allow equilibration of the fiber with the neighboring sediment. The bottom and top of the rods were sealed shut to prevent an inflow of pore water through the system (Lampert et al. in review). The PDMS fibers used in this study were FG 230/210 fibers (Fiber Guide Industries, Stirling, NJ), and had a 210 µm core with a 10 µm PDMS coating or outer diameter of 230 µm.

PAH analysis of the PDMS material was performed at the University of Texas at Austin (UT) using high performance liquid chromatography for separation with fluorescence detection (HPLC/FD) for quantification, in accordance with EPA Method 8310: Polynuclear Aromatic Hydrocarbons using a Waters 2795 Separations Module. Analysis was carried out at the University of Texas, following procedures described by Lampert et al (in prep). The method was optimized for quantification of seven PAHs: phenanthrene (PHE), pyrene (PYR), benz[a]anthracene (BAA), chrysene (CHR), benzo[b]fluoranthene (BBF), benzo[k]fluoranthene (BKF), and benzo[a]pyrene (BAP).

The total organic carbon (f_{oc}) of sediment samples was determined by elemental analysis on a Carlo-Erba 1108 according to Hedges and Stern (1984) modified according to Harris et al. (2001) (i.e., overnight vapor acidification with a hydrochloric acid atmosphere to remove inorganic carbon from samples). The oxidation column was run at 1020°C, while the reduction column was run at 650°C. The oven temperature was maintained at 60°C. Each sample was measured in triplicate and the results averaged to obtain the final values used for analysis.

9.3.6. Passive Samplers- DGT

Diffusive gradients in thin film (DGT) accumulate a variety of dissolved substances, including metals, in a manner similar to uptake by biological organisms. The effective concentration (C_E) measured by DGT

is analogous to the concentration of bioavailable metal, and automatically accounts for all chemical properties of the sampled matrix, including pH, organic carbon, and acid volatile sulfide (AVS). Effective concentration also correlates very well with uptake by biota (Zhang et al. 2001). Commercially available DGT probes consist of a diffusive gel protected by a membrane protected by a plastic housing. These can be inserted into sediments at a desired depth, and are typically retrieved after 1-2 days. The design of these passive samplers facilitates sampling at fine spatial resolutions, as the diffusive gel can be sliced to the desired corresponding sediment depth. Furthermore, because DGTs accumulate dissolved substances over time, they can effectively measure substances at lower concentrations than can be measured using conventional porewater analysis.

The DGT sediment bioassay probes (DGT Research Ltd.) were deployed within SEA Rings at each of the four stations at both NBSD and NAS Pensacola sites. Prior to deployment, DGT probes were placed in a 1 liter acid washed plastic bottle filled with a solution of 0.01 M NaCl plus 5-10 g Chelex-100 in DI, capped, and then nitrogen gas was bubbled into the bottle for 24 h to deoxygenate the probes. DGTs were removed, rinsed in DI and placed into a modified SEA Ring sediment exposure chamber for deployment. Upon recovery of the SEA Rings, DGTs were removed from their holders, and rinsed thoroughly in DI water to remove all traces of sediment. The plastic assembly of each probe corresponding to the sediment-water interface (SWI) was clearly marked using a teflon coated razor, being careful not to contact the probe itself. The SWI was clearly defined for all of the DGT probes. Rinsed and marked probes were placed in labeled plastic ziplock bags containing a small amount of 0.01 M NaCl solution, and refrigerated until ready for processing.

DGT probes were removed from the refrigerator, and DGT gels were carefully removed from their protective plastic casings. For each probe, the top filter membrane and diffusive gel were carefully peeled away, and the remaining resin was placed onto an acid washed glass slab. Using a teflon coated razor blade, six one cm slices (parallel to the sediment-water interface) were made. These six slices included the top 5 cm of the sediment profile (at 1 cm intervals), plus a 1 cm slice just above the sediment-water interface. Each slice was placed in a 2 ml microcentrifuge tube using Teflon coated forceps, to which 1 ml if 1 M HNO_3 was added. Acidified water samples were shipped to an outside laboratory (Alloway Laboratory, Lima, OH) and analyzed for Cu, Zn, Ni, Pb, and Cd using EPA method 200.8 (U.S. EPA 1994). Dissolved metal concentrations were ultimately converted to effective concentrations of labile metal (C_E) using temperature specific diffusion coefficients provided by the DGT manufacturers.

9.3.7. Water quality monitoring

In-chamber water quality was continuously monitored (generally in 30 second intervals) by securing appropriate electrodes inside a representative SED chamber by modifying the top end cap, thus measuring water quality inside the chamber. The sensing device was a Troll 9500 (In Situ Inc., Fort Collins, CO). Measurements included temperature, pH, dissolved oxygen, salinity, conductivity, depth, and ORP. Discrete water samples were also measured in the field following deployment and prior to recovery by removing approximately 30 mL via a sampling port built into the tops of some chambers with a plastic syringe.

9.3.8. Parallel laboratory toxicity testing

Concurrent laboratory toxicity tests were conducted on grab sediment samples, sediment cores, or pore water for both the NBSD and NAS Pensacola studies. For NBSD, parallel testing in the laboratory was conducted using standard methods at SSC Pacific as part of a larger scale concurrent sediment quality evaluation (SSC Pacific, in prep). These methods included 10-day solid phase toxicity tests with the amphipod *E. estuarius*, 48 h sediment-water interface exposures with embryos of the mussel *M. galloprovincialis*, and centrifuged pore water exposures using fertilization success for the purple sea urchin (*Strongylocentrotus purpuratus*). At NAS Pensacola, 96 h and 28 day laboratory sediment

exposures were conducted with *L. plumulosus* and *M. mercenaria* at the US Army Corps Engineer Research and Development Center (Vicksburg, MS).

9.3.9. Ground water discharge assessment at NAS Pensacola

Hydrological characterization tools (Trident Probe and UltraSeep) were employed at NAS Pensacola due to suspected ground water-surface water interactions at the site. The Trident is a direct-push, integrated temperature sensor, conductivity sensor, and pore water sampler that is used to rapidly detect and map areas where groundwater may be discharging to a surface water body. The UltraSeep System provides the ability to directly and continuously quantify ground-water discharge rates and collect flow proportional samples to quantify both water and chemical flux (Chadwick et al. 2003).

Fate and transport of migrating sediment and groundwater contaminants will be assessed with the tools on-board and adjacent to the current Trident and UltraSeep Systems, while effects (bioresponses) from surficial sediments, upwelling groundwater and sediment porewater contaminants, from mobilization of sediment-bound contaminants, or from overlying surface waters, will be assessed concurrently with *in situ* toxicity and bioaccumulation tests (live organisms and passive sampling devices (PSDs).

9.3.10. Pore water toxicity screening

Previous testing on pore water samples as part of this project indicated that this endpoint was at least as sensitive as other more commonly used test organisms, yet is advantageous for pore water screening due to simpler test set up, rapid response time, and small sample volume requirements.

9.3.11. Sediment sampling

Bulk sediment samples collected for laboratory toxicity and bioaccumulation testing and bulk chemistry analysis was conducted from the water surface using a Van Veen grab sampler (NBSD) or with buckets brought down by the divers (NASP, Chollas Creek). In both cases, the top 5 cm was collected, homogenized, split as necessary, and stored at 4 °C until analysis.

9.3.12. Chemical analysis

Bulk sediment, pore water, overlying water, passive samplers and tissues were analyzed for contaminant classes specific using methods outlined in various portions of this report. Tissue analysis of smaller test organisms (i.e. polychaetes, amphipods, small bivalves) was conducted using a micro-extraction method allowing use of relatively small tissue masses (Jones et al. 2006). Method detection or reporting limits are summarized in appropriate sections of the report.

9.3.13. Test sites

A total of three test sites with historically characterized contamination gradients were evaluated in this study for tool development and initial validation. Sites varied to some extent with respect to types of contaminants of concern, sediment and water physico-chemical characteristics, hydrological characteristics, and geographic location. A range of new and emerging technologies together with traditional measures to characterize exposure, uptake and response were incorporated. The stations at each site were selected to demonstrate an anticipated range of responses across the chemical gradient. The overall approach was site-specific, with some measurements, such as those associated with groundwater-surface water interactions (i.e. groundwater discharge rate), being included, based on historical knowledge of the site and synoptically conducted screening assessment (i.e. Trident Probe, pore water toxicity). Table 9-1 lists historical contaminants of concern at each of these test sites and summarizes what components of SEAP were employed as part of each study. Table 9-2 shows the dates and locations of the deployments.

Table 9-1. Summary of measurements made at the three field sites.

Parameter	Naval Base San Diego	NAS Pensacola	Chollas Creek
Historical COCs	metals, PAHs, PCBs, pesticides	metals, PAHs, PCBs, pesticides, VOCs	metals, PAHs, PCBs, pesticides
Lab Toxicity	*E. estuarius* (SED) *M. galloprovincialis* (SWI) *S. purpuratus* (PW)	*L. plumulosus* (SED)	None
In Situ Toxicity	*E. estuarius* (SED) *N. arenaceodentata* (SED) *M. galloprovincialis* (SWI) *A. bahia* (SWI, WC)	*L. plumulosus* (SED) *N. arenaceodentata* (SED) *A. bahia* (SWI, WC)	*E. estuarius* (SED, SWI, WC) *N. arenaceodentata* (SED)
Lab Bioaccumulation	*M. nasuta* (SED)	*M. mercenaria* (SED) *L. plumulosus* (SED)	None
In situ Bioaccumulation	*M. senhousia* (SED) *N. arenaceodentata* (SED)	*M. mercenaria* (SED) *L. plumulosus* (SED)	*M. senhousia* (SED) *E. estuarius* (SED)
SPME	Yes	Yes	Yes
DGT	Yes	Yes	No
In Situ Water Quality	Yes	Yes	Yes
Hydrological Assessment	No	Yes	No

Table 9-2. Date and location of the three sites involving in situ deployment.

Site	Station ID	Date & Time Deployed	Latitude	Longitude
NBSD	NS21	6/17/2008 1217	32.67847	-117.12410
	NS22	6/17/2008 1339	32.67812	-117.12483
	NS24	6/17/2008 1532	32.67702	-117.12692
	CP2243*	6/17/2008 1725	32.66445	-117.14261
NASP	NASP 6B	11/3/2008 1733	30.36351	-87.26807
	NASP 11	11/3/2008 1358	30.36517	-87.26843
	NASP 25	11/3/2008 1759	30.36402	-87.26848
	NASP 9*	11/3/2008 1215	30.36463	-87.26753
Chollas	C14	10/26/2009 1133	32.68756	-117.1298
	C13	10/26/2009 1237	32.68760	-117.13094
	C10	10/26/2009 1457	32.68599	-117.13326
	CP2243*	10/26/2009 1540	32.66452	-117.14278

9.3.13.1. Naval Base San Diego

Naval Base San Diego (NBSD) is the largest Navy base on the west coast of the United Staes, encompassing 13 different piers, and is the principal homeport of 54 ships. Located on San Diego Bay, CA (Figure 9-1), several pier areas at NBSD have been listed as potentially at risk for aquatic life impacts (Fairey et al. 1996; SWRCB 2003). A transect between piers 5and 6 was selected for evaluation of several of the integrated *in situ* assessment tools including short-term toxicity and bioaccumulation testing, with concurrent deployment of both DGTs and SPMEs, due to the historically characterized moderate risk for both metals and non-polar organic contaminants at the site. The *in situ* study was conducted concurrently with a planned large scale laboratory-based spatial assessment of sediment quality (including sediment chemistry, sediment and interstitial water toxicity, benthic community analysis, and bioaccumulation measures) associated with a Phase I TMDL assessment, which included the areas between multiple piers.

9.3.13.2. Naval Air Station Pensacola

An estuarine wetland, the Naval Air Station (NAS) Pensacola Yacht Basin is located at the mouth of Bayou Grande, adjacent to Pensacola Bay, Pensacola, FL. This site was evaluated in a previous remedial investigation (EnSafe 2005, EnSafe 2007) that revealed metals, PAHs, PCBs, DDTs, and VOCs to be of potential ecological risk, particularly at the south end of the water body (Figure 9-2). The potential for groundwater to be a contaminant pathway to surficial sediments and overlying water was also a concern, based on historical data from upstream locations adjacent to the site and the presence of a former landfill bordering the southwest corner of the site. Therefore, in addition to those tools implemented at NBSD, a groundwater discharge zone assessment was also conducted, including both a Trident survey and UltraSeep deployment.

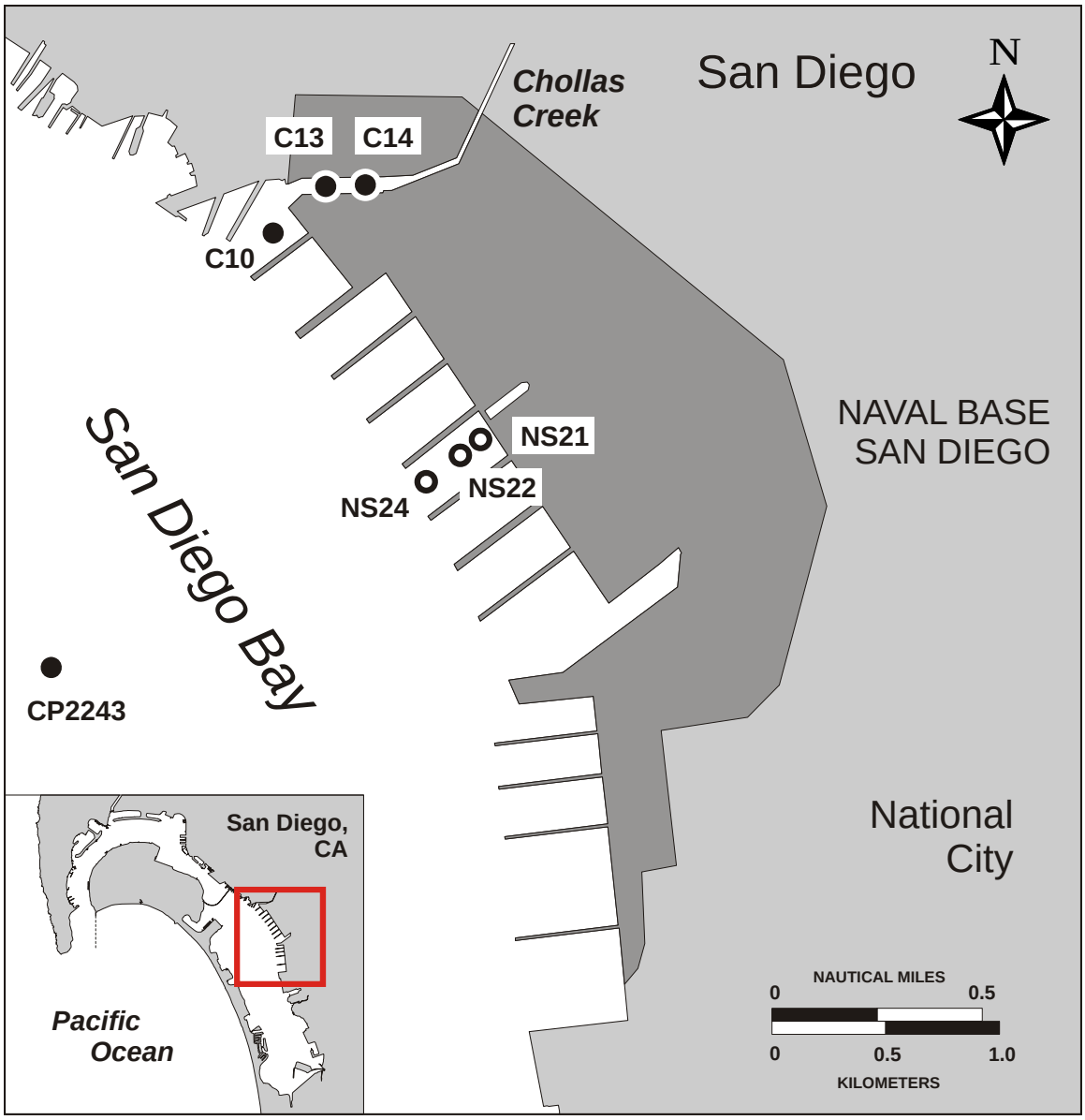

Figure 9-1. Stations bordering San Diego Bay, CA, consisting of two of the test sites. Open circles represent in situ locations evaluated at Naval Base San Diego. Solid circles represent those locations evaluated near the mouth of Chollas Creek. Both studies utilized the same reference station (CP2243).

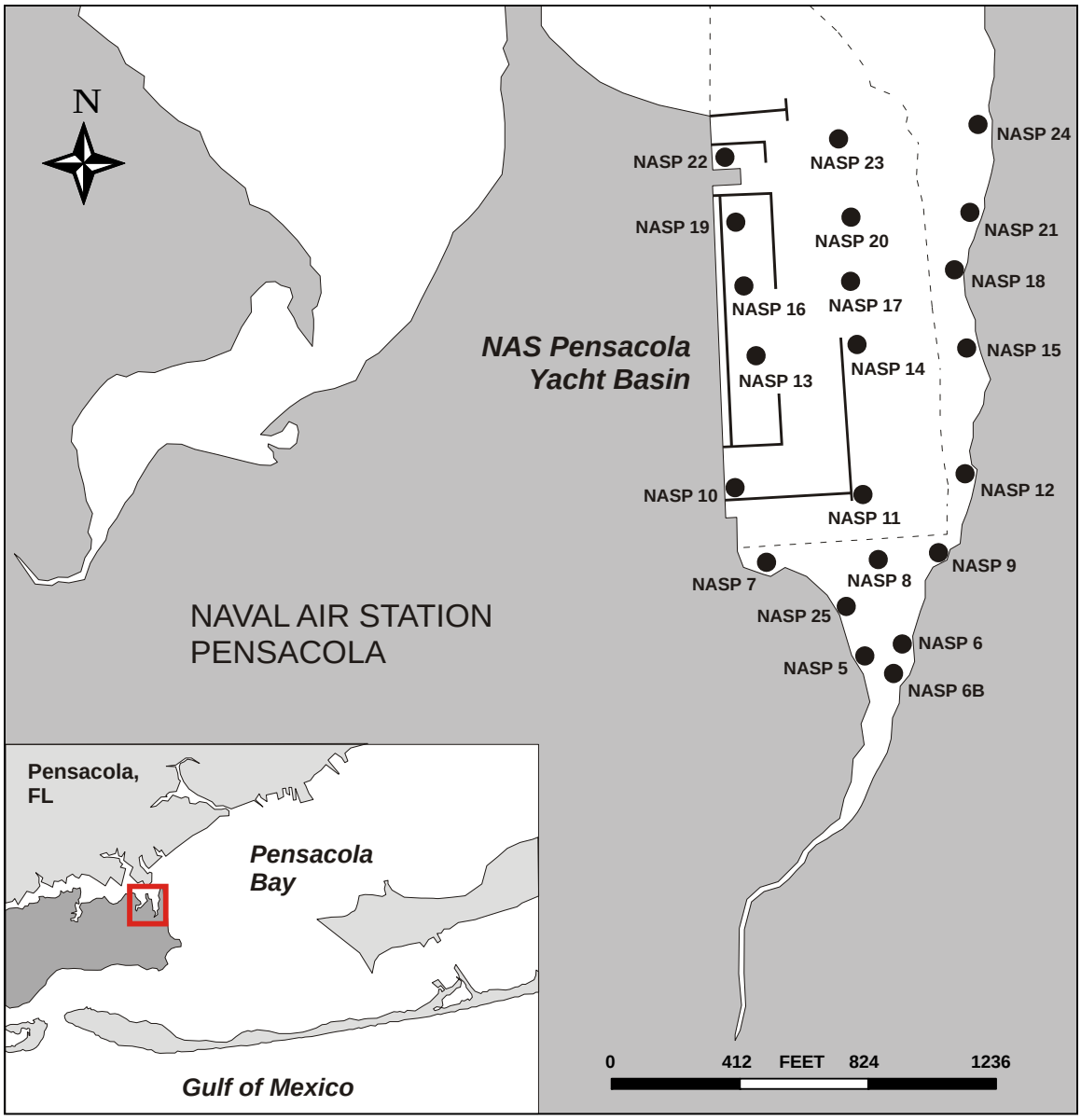

Figure 9-2. Study site at Naval Air Station (NAS) Pensacola showing where Trident Probe was used to locate potential groundwater plumes.

Groundwater Discharge Zone Evaluation. Groundwater discharge was assessed using the Trident and UltraSeep systems (Chadwick et al. 2003). Potential discharge zones were mapped using the Trident conductivity/temperature probe (Figure 9-2). Trident sensor readings were taken at 3 ft below the sediment surface from 26 different stations over a 2-day period. Areas of potential discharge were identified based on low subsurface conductivity. Based on the sensor results, a subset of stations were selected for collection of subsurface porewater samples (annotated by gwd). Porewater samples were collected at 3 ft below the sediment surface. Pore water was also tested on-site for toxicity using the QwikLite 24 h bioassay system. Methods generally followed standard protocol for this test (ASTM 2004). All samples were adjusted to a salinity of 34 ‰ with Crystal Sea MarineMix prior to testing. An UltraSeep was deployed at one station in the discharge zone to quantify discharge rates.

Integrated In-Situ Sediment Assessment. The integrated *in-situ* sediment assessment utilized a range of new and emerging technologies together with traditional measures to characterize exposure, uptake and response at four stations in the wetland. The integrated in-situ sediment assessment involving SEA Rings and the UltraSeep was conducted at four stations that were selected to represent a gradient of contamination, primarily on the basis of results from the Trident/QwikLite surveys, as well as historical data from the site, and included stations NASP6B, NASP9, NASP11, and NASP25 (Figure 9-3). Multiple measures of exposure included bulk sediment chemistry (metals, PAHs, pesticides), porewater, discharge and interface water chemistry (metals, VOCs, PAHs, pesticides), and passive sampler chemistry (metals by DGT, PAHs by SPME). In-situ and laboratory uptake of PAHs was measured for two benthic organisms including *Leptocheirus plumulosus* (marine amphipod) and *Mercenaria mercenaria* (hard clam). In-situ toxicity tests were conducted for three species including *L. plumulosus*, *N. arenaceodentata* (polychaete), and *Americamysis bahia* (mysid shrimp) with parallel lab toxicity testing for *L. plumulosus*. The Sediment Ecotoxicity Assessment Ring (SEA Ring) system was used for passive sampler deployment, as well as in-situ uptake and in-situ toxicity test exposures. Porewater (one foot depth) and interface water samples were collected using the Trident probe. Seepage rates and discharge samples were collected using the UltraSeep. Surface sediment samples were collected by diver deployed cores.

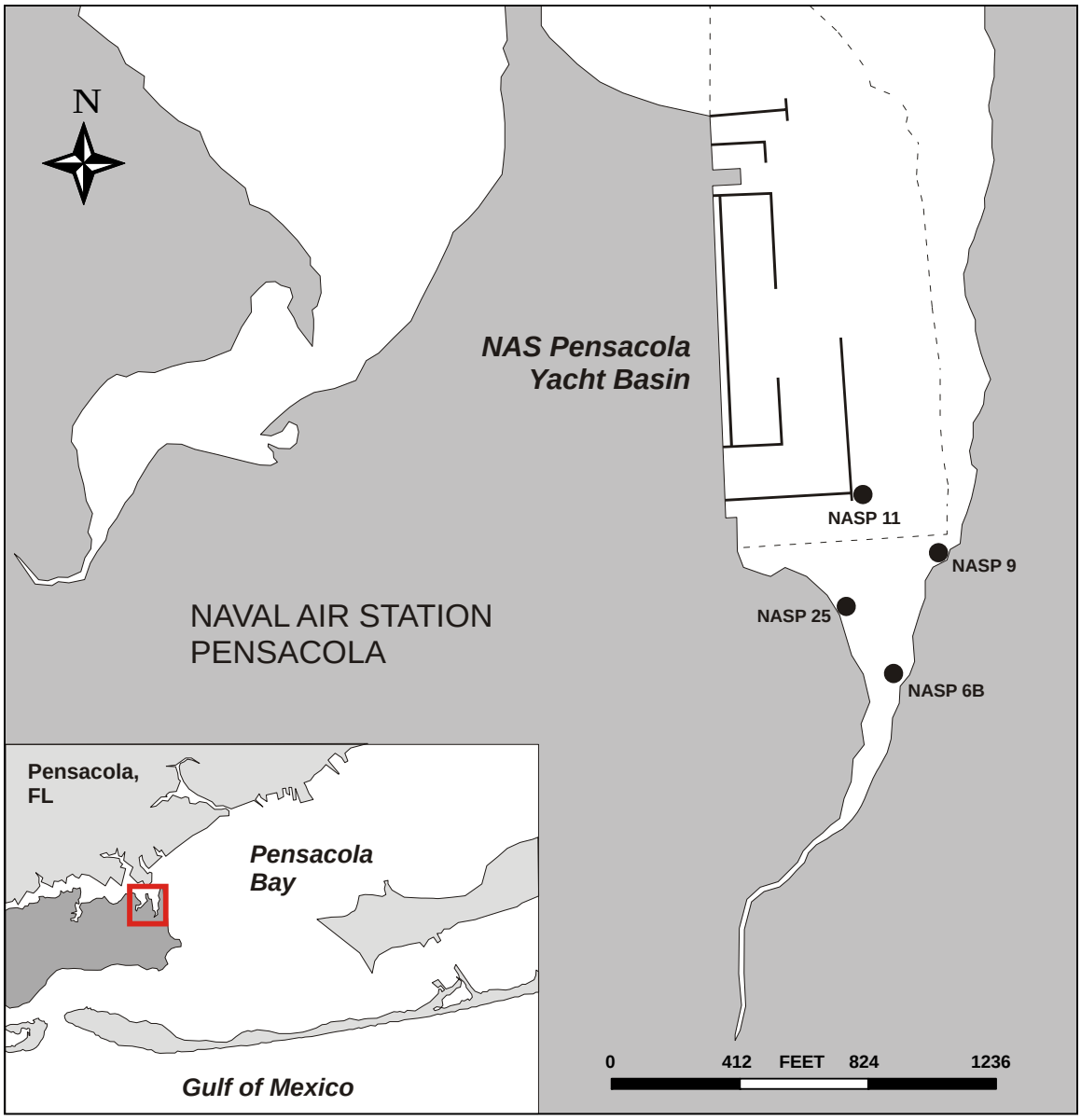

Figure 9-3. Study site at Naval Air Station (NAS) Pensacola showing the location of the four stations selected for in situ assessment.

9.3.13.3. Chollas Creek

The mouth of Chollas Creek borders the north end of NBSD, and is a tributary of San Diego Bay. The site receives upstream inputs from a heavily populated urban part of the City of San Diego, with non-point and point source discharges routinely exhibiting toxicity from both metals (i.e. Cu and Zn) and organophosphate pesticides (Schiff et al. 2002). Lethal and sublethal toxicity and chemical contaminants including metals, PAHs, PCBs, and chlorinated pesticides are chronically elevated in sediments at the mouth of Chollas Creek (Bay and Greenstein 2002, SCCWRP and SSC San Diego 2003, Brown and Bay 2005). The ban of certain organophosphate pesticides has also led to concern about impairment associated with the increased presence of pyrethroid pesticides (Anderson et al. 2010).

The Chollas Creek integrated *in situ* sediment assessment involved SEA Ring deployment at four stations, including one reference station, once again based on an expected contamination gradient. The study examined *in situ* uptake of metals, PAHs, PCBs, and pesticides using benthic organisms including *M. senhousia* and the amphipod *E. estuarius.* Response assays included amphipod (*E. estuarius*) survival in SED, SWI, and WC exposures, and feeding rate reduction by *N. arenaceodentata* in SED exposures. Uptake of PAH in organisms was supplemented by analysis of concurrently deployed PDMS SPME samplers. Samples were also collected for benthic community analyses. Chemical analysis of bulk sediment was also included for the above-mentioned contaminant classes, including pyrethroid pesticides. Passive samplers were analyzed at the University of Texas, while sediment and tissues were analyzed at the USACE ERDC Chemistry laboratory.

Another component of the Chollas Creek study involved experimentation with modifications of the SEA Ring to reduce or eliminated dependence on diver assistance with deployment and recovery. This involved modifications such as the development of a bracket to accommodate aluminum poles (similar to those used to push the Trident Probe) that could be used to deploy the SEA Ring (as well as test organisms), and the use of modified end caps that would remain open during deployment of SED chambers, but close upon recovery to retain the sediment and test organisms.

9.3.14. Data analysis

Means and standard deviations of individual test responses were calculated. Comparisons were made between individual sites and the laboratory control or travel control using unequal variance t-tests, using a level of significance of 0.05. Simple linear regression analysis was used to make comparisons between passive sampler-derived pore water concentrations and tissue, pore water derived from centrifugation, or bulk sediment concentrations. Where relevant, chemical concentrations were compared with sediment quality assessment guidelines (i.e. Long et al. 1995, MacDonald et al. 1996).

9.4. Results

9.4.1. Naval Base San Diego (NBSD)

NBSD Toxicity. Two-day *in situ* exposures resulted in ≥90% survival at the reference site (CP2243) for both *E. estuarius* (amphipod) and *A. bahia* (mysid), while *N. arenaceodentata* (polychaete) mean survival was 75% (Table 9-3, Figure 9-4 and Figure 9-5). A positive correlation (r^2=0.774) was observed between *E. estuarius* mean survival in concurrent two-day *in situ* and 10-day laboratory sediment exposures. Station NS21 was the most toxic to amphipods in both cases (67 and 65% mean survival, respectively), while the reference station sediment induced the least response (Table 9-3, Figure 9-4). Despite the similar responses, the *in situ* exposures did not result in statistically lower ($p<0.05$) survival relative to the laboratory controls, while the laboratory exposures did.

N. arenaceodentata post exposure feeding rate following 2-day exposure in SED chambers was also lowest at station NS21, followed by NS24, NS22, and the reference station (CP2243; Figure 9-5). Because the laboratory and travel controls differed to some degree, statistical comparisons were made with both controls. All stations, including the reference station, resulted in statistically lower feeding rates than the laboratory control, while only station NS21 and NS24 were statistically lower relative to the travel control.

Table 9-3. Results of in situ and laboratory toxicity tests conducted at Naval Base San Diego. Dashes indicate not tested. Bold values indicate staistically significant from control (p<0.05). indicates value statistically different from control, but not considered toxic using the minimum significant difference criterion (Phillips et al., 2001).

Species	*E. estuarius*		*E. estuarius*		*N. arenaceodentata*		*N. arenaceodentata*		*A. bahia*	
Location	Lab		*In Situ*		*In Situ*		*In Situ*		*In Situ*	
Exposure Type	SED		SED		SED		SED		WC	
Endpoint	% Survival		% Survival		% Survival		Feeding Rate[1]		% Survival	
Sample ID	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lab Control	88	8.4	96	9.0	100	0.0	157	5.8	100	0
Travel Control	-	-	93	5.0	100	0.0	135	23.5	100	0
NS21	**65**	13.7	67	27.5	88	15.0	**101**	19.4	100	0
NS22	**75**	10.6	79	14.9	**75**	12.9	129	18.7	100	0
NS24	**77**	5.7	70	21.6	**75**	17.3	**105**	19.3	100	0
CP2243	88	6.7	90	7.1	**75**	17.3	136	7.8	98	5

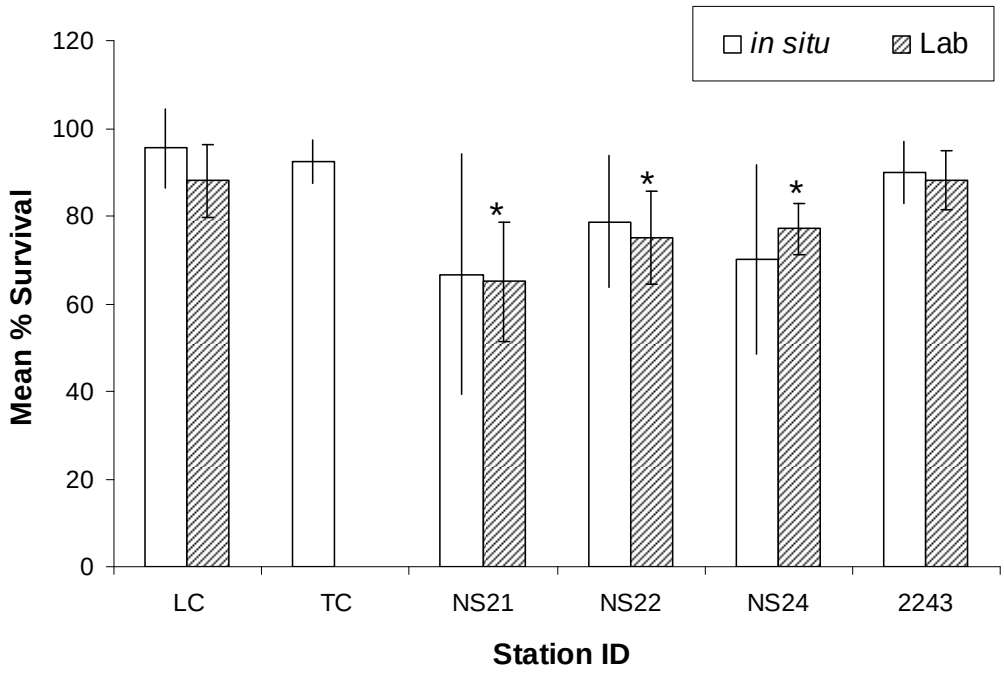

Figure 9-4. Comparison of amphipod (*E. estuarius*) survival following 2-day in situ and 10-day laboratory exposures to surficial sediment at Naval Base San Diego. LC=laboratory control. TC=travel control. Asterisks indicates statistically different from laboratory control (p<0.05). N=5.

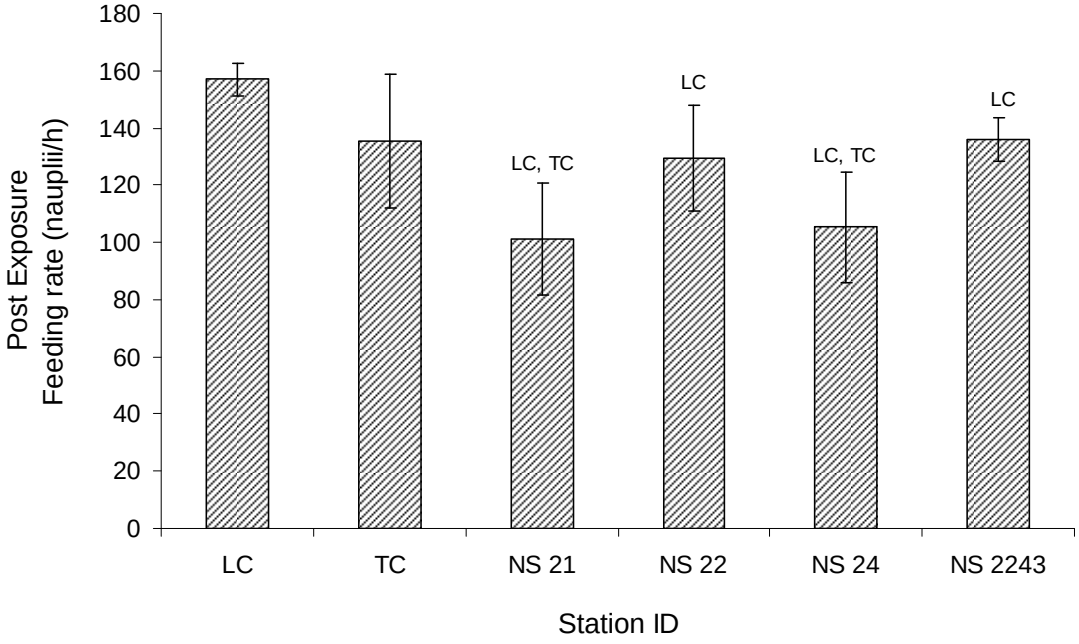

Figure 9-5. Comparison of polychaetes (*Neanthes arenaceodentata*) feeding rate in the laboratory following a 48-h in situ exposure in surficial sediments at Naval Base San Diego. LC=laboratory control. TC=travel control.

Table 9-4. Results from *M. galloprovincalis* embryo-larval development tests conducted in situ at the sediment-water interface at Naval Base San Diego. LC=laboratory control.

	# Normally Developed		% Normal Alive	
Treatment	Mean	SD	Mean	SD
LC	262	24.0	91.1	8.4
NS21	28.5	25.4	9.9	8.8
NS22	14.5	13.5	5.1	4.7
NS24	44.3	9.1	15.4	3.2
NS2243	25.7	20.7	8.9	7.2

Recovery of surviving *A. bahia* from 2-day WC exposures was very high (≥98%) at all stations (Table 9-3). *M. galloprovincialis* larval recovery from *in situ* SWI exposure was low and extremely variable for all in situ stations including the reference site (Table 9-4). A notable absence of toxic effects in laboratory SWI exposures using intact core samples, however, was observed for all stations for this endpoint (SSC Pacific, in prep).

NBSD Water Quality. Continuously measured water quality results from the *in situ* exposures at NBSD are summarized in Table 9-5). All parameters were within those tolerated by the test species, and were relatively close to the laboratory testing water quality conditions (SSC Pacific, in prep).

Bulk Sediment and Pore water Chemistry. Table 9-6 shows a summary of the bulk sediment concentration for metal and organic contaminants of concern. Figure 9-6 depicts contamination gradients for copper and total PAHs at NBSD.

NBSD Bioaccumulation. Organisms were deployed for bioaccumulation assessment for 2 and 21 days. The 21 day deployment of the infaunal mussel (*M. senhousia*) that is resident in San Diego Bay, and N. arenaceodentata, yielded satisfactory survival (74 and 42% overall, respectively) for body burden determinations. PAH body residues from 21-day in situ exposures with *M. senhousia* are shown in Table 9-7.

Table 9-6. Summary of relevant contaminants measured in bulk sediments during in situ deployment at each of the three study sites. ERL=Effects range low; ERM=Effects range median (Long et al. 1995). *indicates that for BHC, sediment quality guidelines are based on TEL and PEL values (MacDonald et al. 1996). < indicates values were below both method detection limit and reporting limit. Italicized values were above method detection limit, but below reporting limit. Dash indicates measurement not made for that station/sample. N/A indicates no value available.

Total PAH concentrations were highest at station NS21, and decreased in the following order: NS24>NS22>NS2243 (reference station). PCBs were measured following 2-day exposures in *N. arenaceodentata* (Figure 9-7) and both *N. arenaceodentata* and *M. senhousia* in 21-day exposures (Figure 9-8). Total PCB concentration after two days of exposure were greater than 21-day concentrations by a factor of two to four. PCB uptake was negligibly elevated relative to the background concentrations and the reference site for *M. senhousia.*

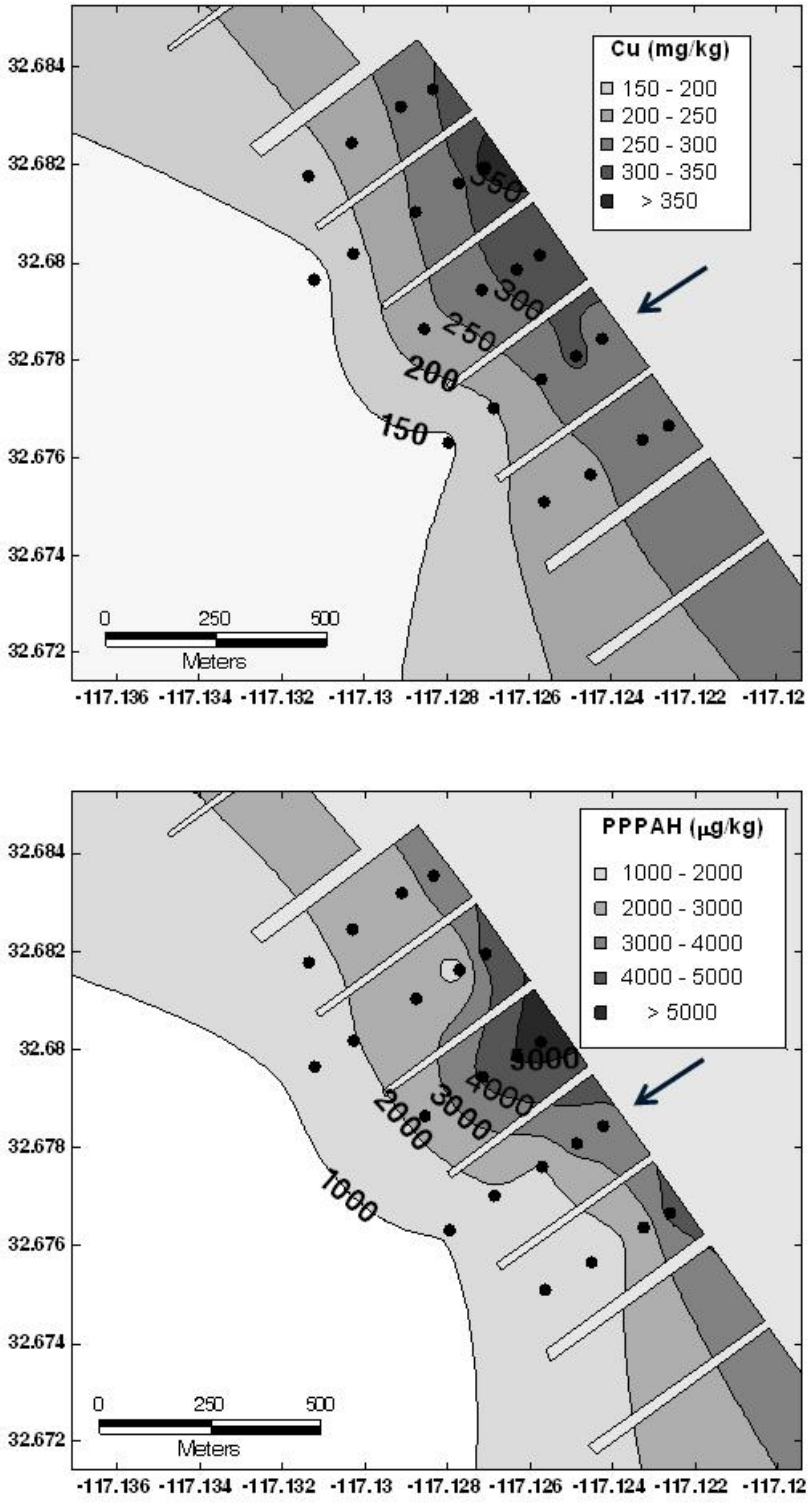

Figure 9-6. Representative bulk sediment chemical concentrations, illustrating contamination gradient observed during the in situ study at Naval Base San Diego (top=copper; bottom=priority pollutant PAHs). Arrow points to area where in situ work was conducted.

Table 9-5. Water quality parameters expressed as mean (minimum, maximum) from each of the three field deployments. Water quality was measured with a Troll 9500 (In Situ, Inc.) instrument positioned inside a representative sediment chamber just above the sediment-water interface at 30 second intervals. Dashes indicate technical error that prevented the sensor from collecting data for that parameter.

Site	Station	Temperature (ºC)	Depth (m)	Dissolved oxygen (mg/L)	pH	Salinity (ppt)	Conductivity (mS/cm)	ORP (mV)
NBSD	NS21-1	22.3 (21.4, 22.8)	10.5 (9.4, 11.6)	5.7 (4.0, 7.1)	6.8 (6.8, 7.0)	35.7 (35.1, 36.1)	51.2 (49.7, 52.1)	84 (-156, 164)
	NS22-1	22.2 (21.3, 22.8)	10.2 (9.1, 11.3)	4.9 (3.1, 7.3)	7.6 (7.3, 7.9)	33.1 (32.7, 33.3)	47.8 (46.4, 48.5)	194 (-72, 254)
	NS24-1	22.6 (21.7, 23.3)	11.2 (10.0, 12.3)	5.6 (4.3, 6.4)	7.7 (7.4, 7.9)	34.0 (33.4, 34.4)	49.3 (47.7, 50.5)	159 (-84, 234)
	CP2243*	23.1 (22.5, 23.7)	4.7 (3.5, 5.8)	7.1 (6.5, 8.2)	7.9 (7.7, 8.0)	33.4 (31.7, 36.0)	49.0 (46.8, 52.6)	-
NASP	NASP 6B	20.2 (19.3, 22.2)	0.6 (0.1, 0.9)	8.1 (5.5, 10.3)	7.1 (7.0, 7.3)	16.0 (15.6, 16.4)	23.7 (23.3, 24.2)	-347 (-371, -167)
	NASP 11	19.6 (18.9, 20.4)	2.5 (2.2, 2.7)	4.9 (1.0, 8.2)	7.8 (7.4, 8.1)	25.1 (24.7, 25.5)	35.2 (34.2, 36.2)	232 (133, 319)
	NASP 25	20.2 (19.2, 21.3)	0.7 (0.4, 1.0)	7.8 (3.3, 11.1)	7.6 (7.5, 7.7)	23.5 (22.4, 24.0)	33.7 (33.3, 34.6)	-233 (-283, -162)
	NASP 9*	20.0 (18.2, 21.6)	0.7 (0.4, 1.0)	6.5 (3.8, 8.7)	8.0 (7.6, 8.2)	24.7 (23.1, 24.7)	34.2 (32.2, 35.9)	116 (54, 133)
Chollas	C14	21.5 (20.6, 22.1)	2.3 (1.6, 2.9)	3.0 (-0.1, 5.7)	-	-	-	-
	C13	-	-	-	-	-	-	-
	C10	21.9 (21.5, 22.0)	10.9 (10.2, 11.5)	6.1 (2.5, 6.6)	7.9 (7.8, 7.9)	31.1 (28.5, 39.7)	44.9 (41.5, 55.4)	208 (101, 287)
	CP2243*	21.1 (20.2, 22.4)	4.7 (4.1, 5.3)	6.2 (2.6, 7.6)	7.9 (7.8, 8.0)	35.2 (34.4, 36.2)	49.4 (48.0, 50.1)	221 (120, 297)

Table 9-6. Summary of relevant contaminants measured in bulk sediments during in situ deployment at each of the three study sites. ERL=Effects range low; ERM=Effects range median (Long et al. 1995). *indicates that for BHC, sediment quality guidelines are based on TEL and PEL values (MacDonald et al. 1996). < indicates values were below both method detection limit and reporting limit. Italicized values were above method detection limit, but below reporting limit. Dash indicates measurement not made for that station/sample. N/A indicates no value available.

				Site/Station											
				NBSD				NASP				Chollas Creek			
Analyte	Units	ERL	ERM	NS21	NS22	NS24	CP2243*	NASP 6B	NASP 11	NASP 25	NASP 9*	C14	C13	C10	CP2243*
Cd	mg/kg	1.2	9.6	0.354	0.267	0.669	0.136	15.9	2.72	18.2	0.936	0.183	0.837	0.314	0.117
Cr	mg/kg	81	370	81.1	94.5	79.7	48.0	520	90.7	523	32.9	9.2	27.9	45.7	29.0
Cu	mg/kg	34	270	277	316	197	79.8	66.7	25.6	230	9.64	13.8	99.4	208	69.6
Hg	mg/kg	0.15	0.71	0.76	0.91	0.79	0.35	0.23	0.45	0.97	BDL	0.01	0.19	0.53	0.323
Pb	mg/kg	46.7	218	73.9	75.4	63.2	33.3	226	35.7	326	15.0	27.1	69.8	73.2	26.4
Zn	mg/kg	150	410	342	338	308	159	229	62.1	396	21.8	83.0	292	266	139
g-BHC (Lindane)	µg/kg	0.32*	0.99*	<0.25	<0.25	<0.25	<0.25	*5.09*	<5.5	*5.38*	<4.7	<0.13	<0.13	<0.13	<0.13
Tot Chlordane	µg/kg	0.5	6	0.600	0.420	0.820	0.04	*3.74*	<5.5	<12.3	<4.7	<0.12	<0.12	<0.12	<0.12
Tot DDX	µg/kg	1.58	46.1	6.32	6.62	7.41	0.875	73.2	2.24	26.7	<4.7	2.89	9.03	11.1	2.63
Permethrin	µg/kg	N/A	N/A	-	-	-	-	-	-	-	-	<0.7	15.7	<0.7	<0.7
Tot PAH	µg/kg	4022	44792	5214	5105	2924	415	13392	973	10114	667	444	2043	1012	225
Tot PCB	µg/kg	22.7	180	234	172	207	21.4	-	-	-	-	43.1	104	79.0	16.6
Silt/Clay	**%**			72.5	85.9	67.1	40.9	19.8	19.7	53.1	11.7	11.8	68.6	69.7	39.8
TOC	**%**			2.01	2.14	1.58	0.710	5.95	1.46	7.63	0.627	0.972	3.56	2.65	0.995

Table 9-7. PAH body residues (µg/kg) measured in the infaunal mussel *Musculista senhousia* following 21-day in situ exposure at Naval Base San Diego. BDL=below detection limit. Method Detection Limit = 0.01 µg/kg.

	M. senhousia									
	Time 0		NS 21		NS 22		NS 24		NS 2243	
Analyte	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2-Methylnaphthalene	BDL	-	BDL	-	BDL	-	BDL	-	BDL	-
Acenaphthene	BDL	-	BDL	-	BDL	-	BDL	-	BDL	-
Acenaphthylene	BDL	-	BDL	-	BDL	-	BDL	-	BDL	-
Anthracene	BDL	-	BDL	-	BDL	-	BDL	-	BDL	-
Benzo (a) anthracene	BDL	-	BDL	-	BDL	-	BDL	-	BDL	-
Benzo (a) pyrene	BDL	-	**10.84**	**9.43**	BDL	-	**10.06**	**11.73**	BDL	-
Benzo (b) fluoranthene	BDL	-	**25.00**	**5.96**	**6.71**	**11.60**	**28.95**	**7.76**	BDL	-
Benzo (g,h,i) perylene	BDL	-	BDL	-	BDL	-	BDL	-	BDL	-
Benzo (k) fluoranthene	BDL	-	**17.50**	**5.20**	BDL	-	**10.06**	**11.73**	BDL	-
Chrysene	BDL	-	**9.20**	**8.08**	BDL	-	**14.80**	**7.40**	BDL	-
Dibenz (a,h) anthracene	BDL	-	BDL	-	BDL	-	BDL	-	BDL	-
Fluoranthene	BDL	-	**17.50**	**5.20**	BDL	-	BDL	-	BDL	-
Fluorene	BDL	-	BDL	-	BDL	-	BDL	-	BDL	-
Indeno (1,2,3-cd) pyrene	BDL	-	BDL	-	BDL	-	BDL	-	BDL	-
Naphthalene	BDL	-	BDL	-	BDL	-	BDL	-	BDL	-
Phenanthrene	BDL	-	BDL	-	BDL	-	BDL	-	BDL	-
Pyrene	BDL	-	**17.50**	**5.20**	BDL	-	**8.21**	**9.55**	BDL	-
Total PAH	BDL	-	97.54	39.09	6.71	11.60	72.07	48.15	BDL	BDL

NBSD DGTs. Effective concentrations (C_E) in sediments were highest for zinc (Zn), followed in order of decreasing concentration by copper (Cu), nickel (Ni), lead (Pb), and cadmium (Cd) (Figure 9-9, Table 9-8). With the exception of station NS21, Cd was detected only in the 1cm of the water column above the sediment water interface. With the exception of Cu, metal concentrations at the reference site (CP2243) were lower than all three of the test sites (Table 9-8). In contrast, Cu concentrations were slightly higher (1.55 µg/l) at the reference station than at station NS24 (1.20 µg/l) and NS22 (1.11 µg/l), and about one half as high as station NS22 (3.07 µg/l). Metal concentrations measured by DGTs were generally similar (Cu, Cd) or lower (Zn, Ni, Pb) than porewater (collected by centrifugation) metal concentrations measured at the same locations.

Table 9-8. Concentrations (µg/l) of metals measured using diffusive gradient thin films (DGT) probes (top) and measured from porewater (bottom), San Diego Bay, CA, June 2008. Reported concentrations from DGT probes are averaged across the top 5 cm of the sediment.

Site	Cu	Zn	Ni	Pb	Cd
DGT Effective Concentration (µg/l)					
NS2243	1.551	4.225	0.208	0.041	0
NS24	1.198	10.25	0.278	0.463	0
NS22	1.106	16.32	0.394	0.070	0
NS21	3.067	13.54	0.489	0.189	0.021
Porewater Concentration (µg/l)					
NS2243	1.19	5.22	2.13	0.128	0.008
NS22	1.55	57.4	1.46	0.353	0.026

No distinct spatial patterns were evident within the sediment profiles, although there did appear to be an overall trend of decreasing Ni concentrations with depth at all stations, and decreasing Cu concentrations at all stations except for NS21. Concentrations of Pb and Zn also increased with depth at station NS21; however, concentrations of Cd and Ni decreased with increasing depth.

Coefficients of determination (r^2) for bulk sediment concentration and C_E were as follows: Cu (0.036); Zn (0.874); Pb (0.070); Cd (0.001); and Ni (0.828).

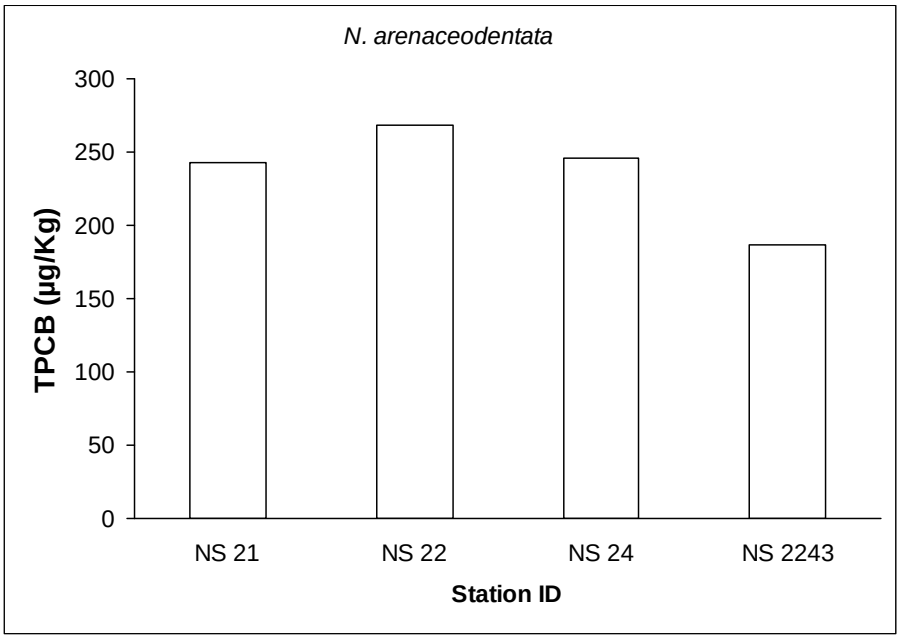

Figure 9-7. Total PCB concentrations following 2-day in situ deployment of polychaetes (Neanthes arenaceodentata) at Naval Base San Diego. Worms were composited from several chambers for a total of one sample per station.

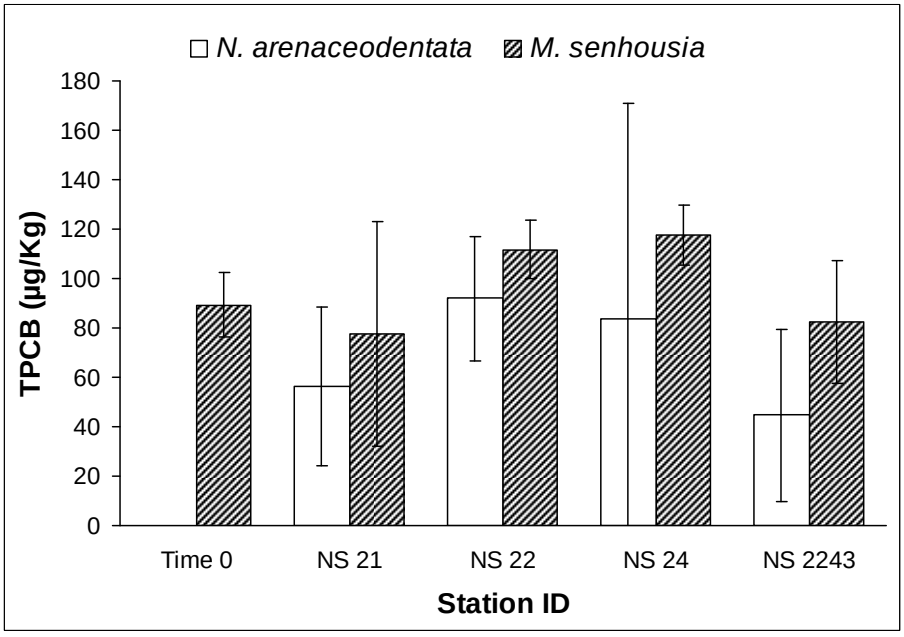

Figure 9-8. Mean (± 1 s.d.) total PCB concentrations following 21-day *in situ* deployment of infaunal bivalve (*Musculista senhousia*) and polychaetes (*Neanthes arenaceodentata*) at Naval Base San Diego. N=4.

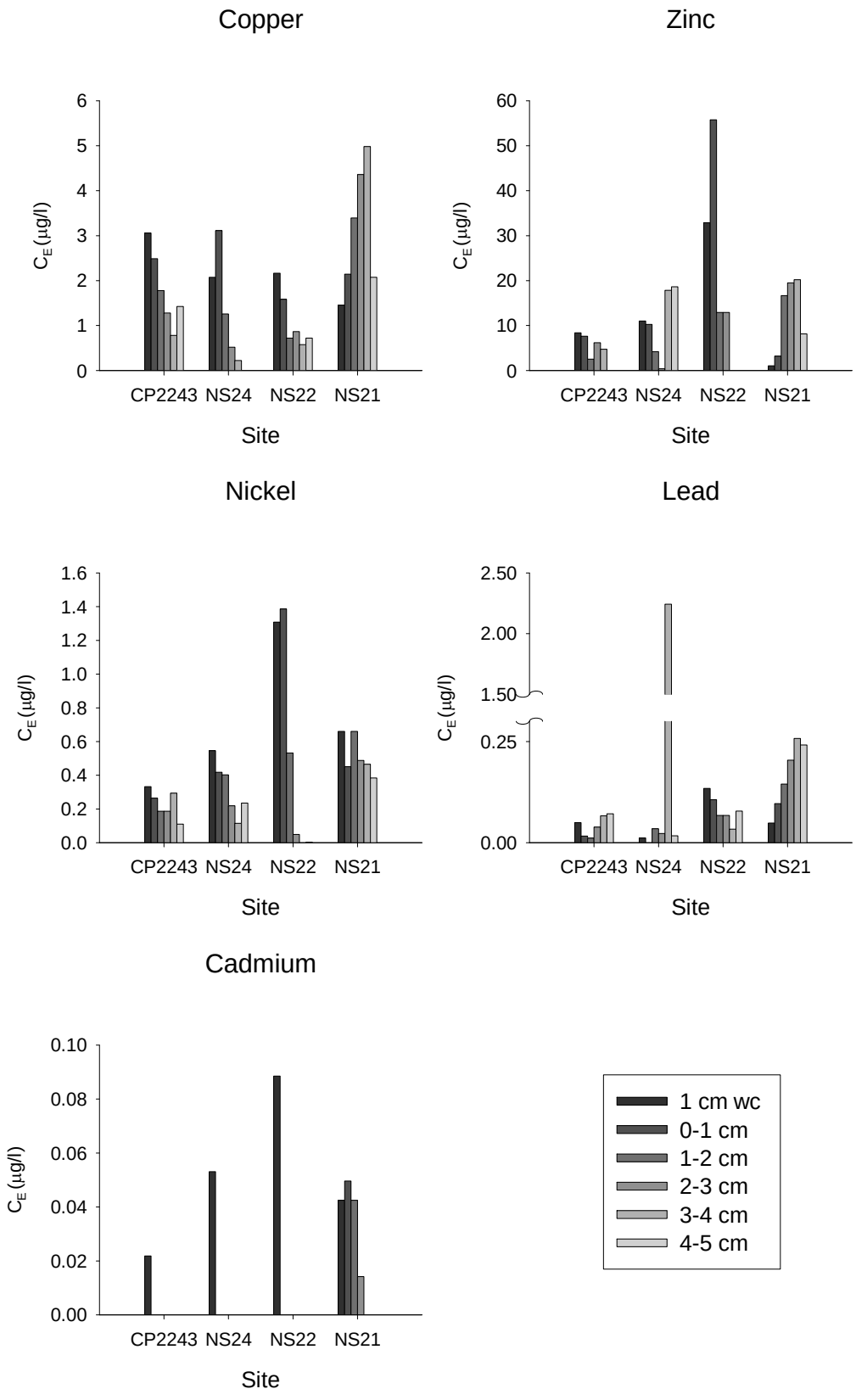

Figure 9-9. DGT results for NBSD.

NBSD SPME. Figure 9-10 shows a plot of the measured tissue concentrations for three PAHs (BbF, BkF, and BaP) versus organic carbon-normalized solid-phase concentrations, pore water concentrations derived from centrifugation, and PDMS-derived pore water concentrations, along with linear fits to the data. The concentrations of other PAHs were below detection limits. Because the values of Kow (6.1 to 6.13) are similar for each of these compounds, the data for each of the individual compounds can be combined for analysis. As with the toxicity and PAH uptake by *M. senhousia*, PDMS-derived pore water concentrations were highest for NS21, followed in decreasing order by NS24, NS22, and CP2243.

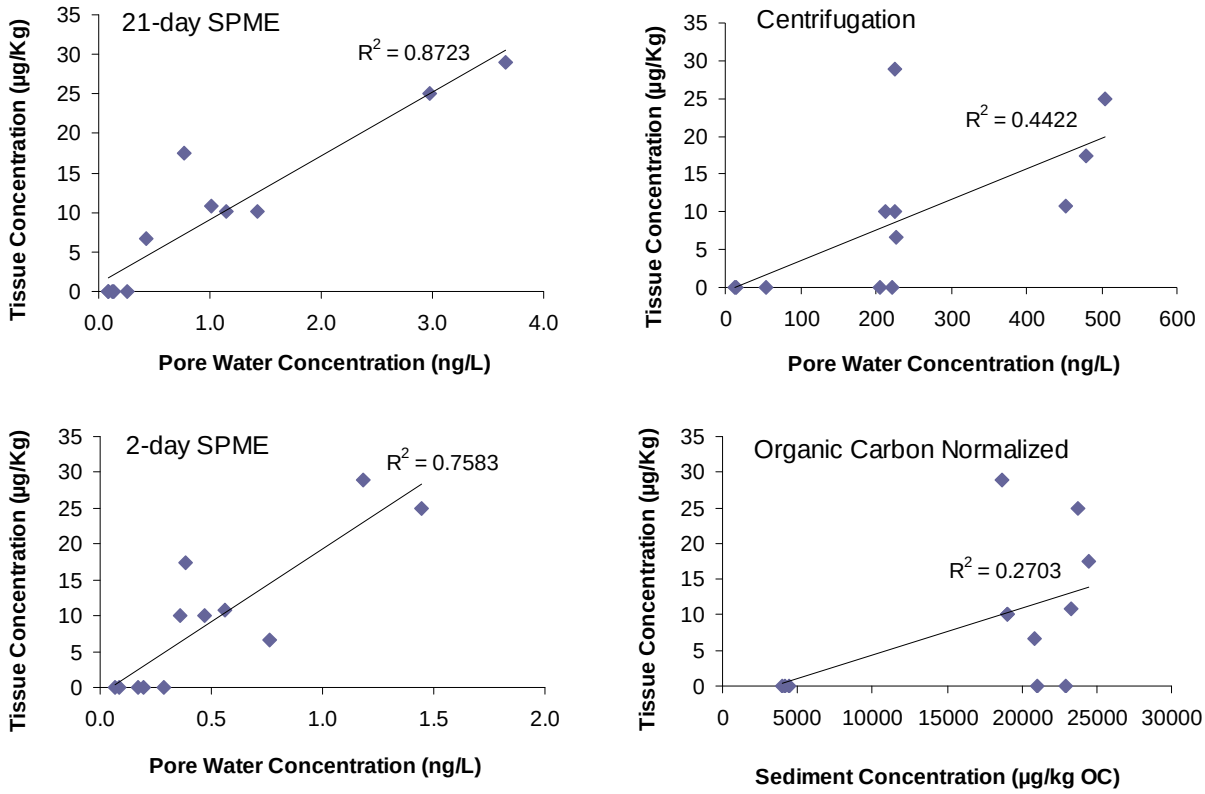

Figure 9-10. Uptake of PAHs from 21-d *in situ* exposures with the infaunal mussel *Musculista senhousia* at Naval Base San Diego relative to 21-day SPME derived pore water concentration (top left); 2-day SPME derived pore water concentration (bottom left); centrifugation-derived pore water concentration (top right); and organic carbon normalized sediment concentration (bottom right). Because of their similar hydrophobicities, data from the three PAHs (BbF, BkF, BaP) are shown combined.

9.4.2. NAS Pensacola

9.4.2.1. NAS Pensacola ground water discharge assessment

Trident Sensor Survey. The results of the Trident sensor survey are shown in Figure 9-11**,** Subsurface conductivity results indicated that the strongest evidence of groundwater discharge was along the near-shore areas adjacent to the former landfill (southwest corner of the water body), particularly in the area of NASP5, NASP25, and NASP26. An isolated instance of low conductivity was also observed further north along the marina shoreline at NASP 10, however, this location was remote from known sources of groundwater contamination.

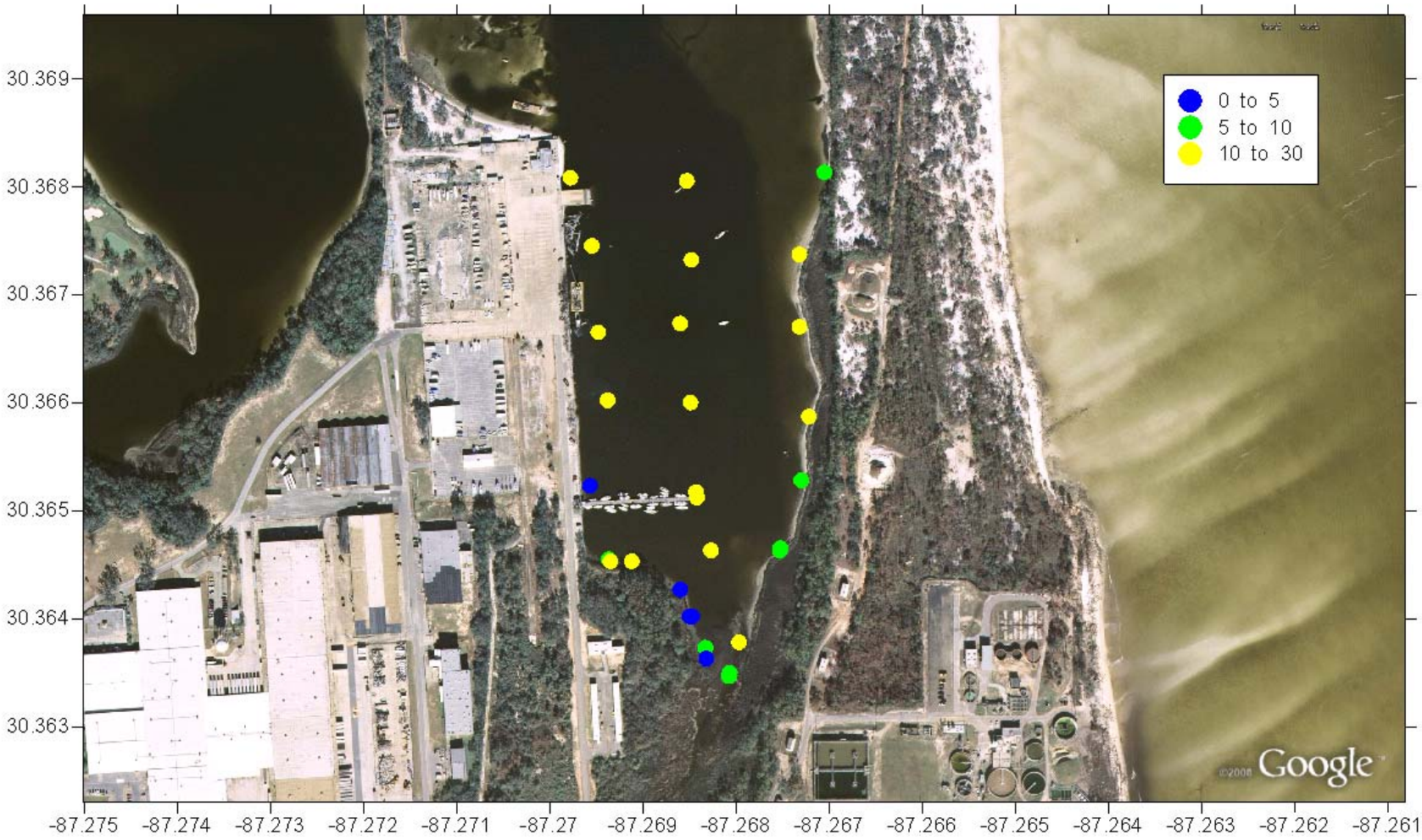

Figure 9-11. Trident sensor survey results for subsurface conductivity (mS/cm).

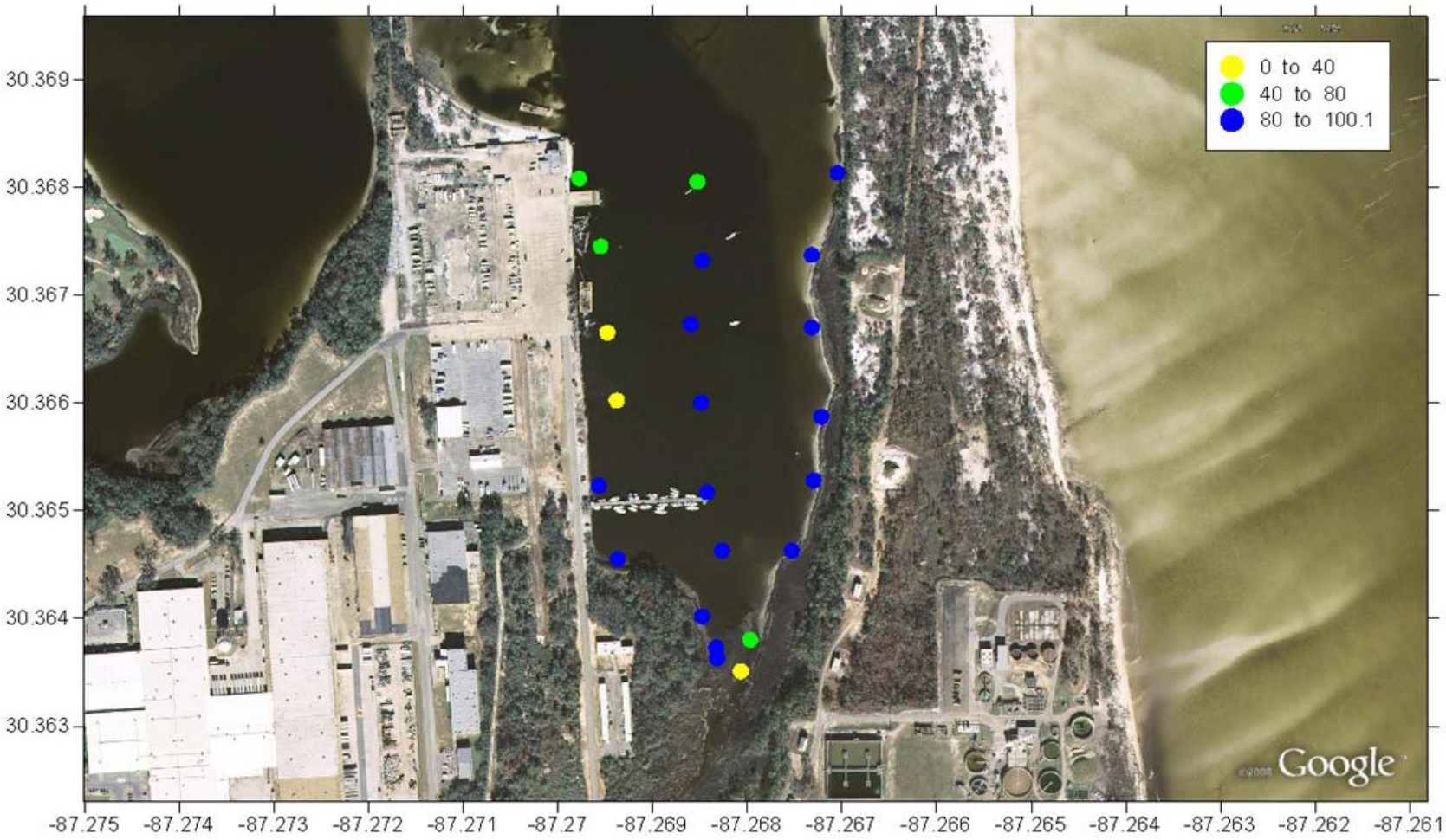

Figure 9-12. QwikLite screening results at NAS Pensacola.

Based on the sensor results, five stations (NASP5, NASP7, NASP25, NASP26, NASP27; Figure 9-2) were selected in proximity to the former landfill for collection of subsurface porewater samples. These samples were analyzed for VOCs, which were generally below reporting limits for all analytes at all stations. Hexachlorobutadiene was detected below the reporting limit at stations NASP5 and NASP7, but was detected in water blanks at comparable levels. Naphthalene and 1,2,4-Trichlorobenzene were detected below reporting limits at station NASP 5.

Pore water toxicity screening. Pore water samples from the Trident survey were tested on-site with the 24 h QwikLite assay, comparatively examined in Task 1 of this project (see Section 4.0). Several stations initially showed a significant response along the southern shoreline, as well as near the quay wall along the northwest corner of the Yacht Basin (Figure 9-12). The majority of the stations that exhibited toxicity (<80% light output relative to control), however, were characterized by having low dissolved oxygen concentration (<2 mg/L), and relatively high total ammonia (>8 mg/L). Therefore, these samples were re-run following vigorous aeration for one hour. Aeration provided satisfactory water quality for the duration of the exposure, and all re-run samples except NASP6B and NASP 16 resulted in a substantially reduced response relative to unaerated samples.

UltraSeep Survey. An UltraSeep was deployed at station NASP25 to quantify the rate of groundwater seepage in the discharge zone identified by the Trident. Seepage rates were measured over a 24 h period and results are shown in Figure 9-13 along with the tidal variation during the deployment period. The seepage rate varied from about -0.8 cm/day (recharge), to about +2.9 cm/day (discharge), with strongest discharge in phase with low tide conditions. The mean discharge rate for the 24 hour period was 0.9 cm/day. No VOCs were detected in the discharge water collected by the UltraSeep.

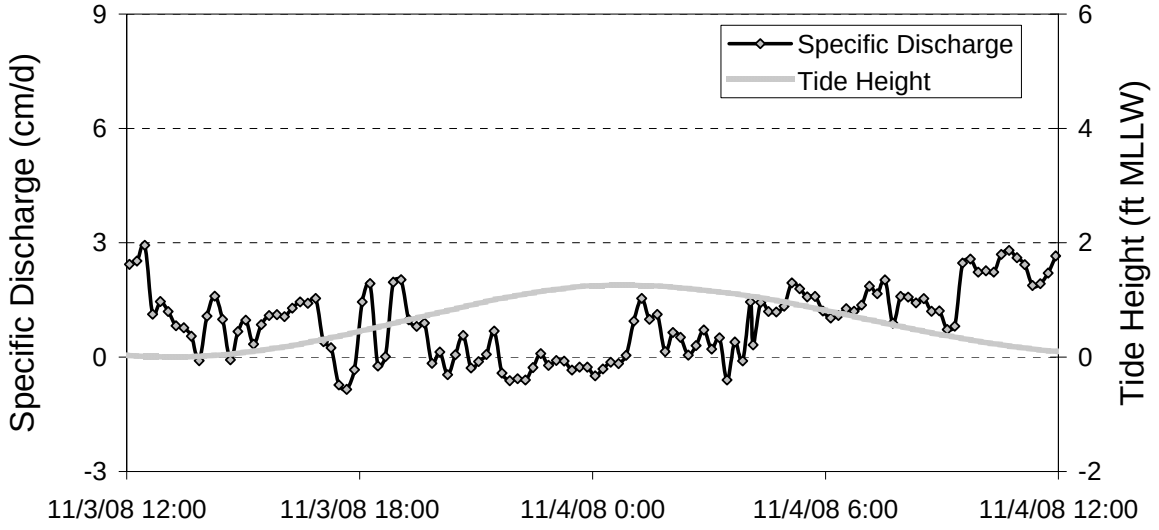

Figure 9-13. Specific discharge and tide stage at station NASP25, at NAS Pensacola.

9.4.2.2. Integrated in situ assessment at NAS Pensacola

NAS Pensacola In Situ Toxicity. Responses from *in-situ* and laboratory toxicity tests for *L. plumulosus, N. arenaceodentata*, and *A. bahia* are shown in Table 9-9 and Figure 9-14 through Figure 9-17. High control survival (≥ 92%) was observed in both lab and *in situ* toxicity exposures for all species, except *N. arenaceodentata*, for which survival was not assessed. Effects were not observed in *in situ* tests conducted in WC or SWI chambers. Amphipod survival was significantly lower ($p<0.05$) at one station

(NASP 6B) relative to the controls in both the *in situ* and lab tests. *In situ* survival (50%) at NASP 6B, however, was considerably lower than in the lab (85%) from the 4-day toxicity exposures. Observed responses did not differ much between 48 and 96 h of exposure. The relatively low mean feeding rate response for *N. arenaceodentata* at NASP 6B suggests toxicity based on this endpoint, but the reduction was not statistically significant.

Table 9-9. Results of in situ and laboratory toxicity tests conducted at NAS Pensacola. Dashes indicate not tested.

	Species	*L. plumulosus*		*L. plumulosus*		*A. bahia*		*A. bahia*		*N. arenaceodentata*	
	Location	Lab		*In Situ*		*In Situ*		*In Situ*		*In Situ*	
	Exposure Type	SED		SED		WC		SWI		SED	
	Endpoint	% Survival		% Survival		% Survival		% Survival		Feeding Rate*	
Duration	**Sample ID**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
48 hours	Lab Control	-	-	98	5.0	98	5.0	98	5.0	83	18
	Travel Control	-	-	94	6.3	88	9.6	88	9.6	77	13
	NASP 9	-	-	89	11.1	100	0.0	100	0.0	79	11
	NASP 25	-	-	80	18.0	100	0.0	100	0.0	80	6
	NASP 6B	-	-	**35**	**15.8**	93	5.8	93	5.8	67	30
	NASP 11	-	-	90	8.2	100	0.0	100	0.0	91	8
96 hours	Lab Control	92	3.0	98	5.0	98	5.0	98	5.0	-	-
	Travel Control	NA	NA	94	6.3	88	9.6	88	9.6	-	-
	NASP 9	92	3.0	81	16.5	98	5.0	90	8.2	-	-
	NASP 25	94	5.3	87	10.4	97	5.8	93	5.8	-	-
	NASP 6B	**85**	**5.0**	**50**	**13.2**	100	0.0	87	23.1	-	-
	NASP 11	93	3.0	87	7.6	93	5.0	88	15.0	-	-

Bold indicates statistically lower than associated Lab or Travel Control using unequal variance t-tests ($p<0.05$).
SED=surficial sediment; WC=water column; SWI=sediment-water interface
*Number of brine shrimp nauplii consumed in equivalent of one hour following a 48 hour sediment exposure.

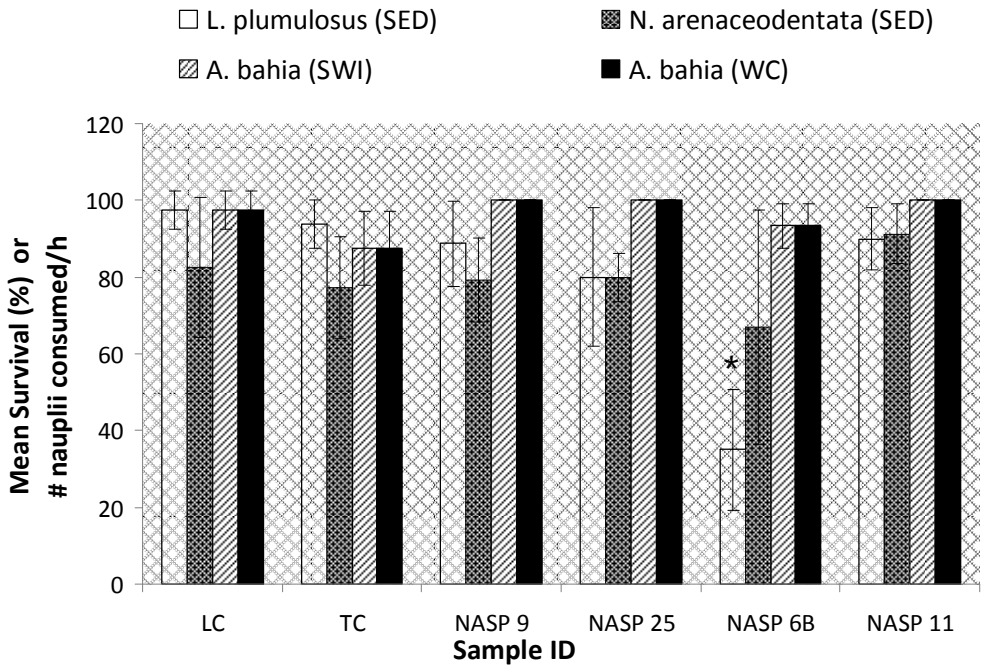

Figure 9-14. Results from 48 h in situ toxicity tests at NAS Pensacola. Response was percent survival for *L. plumulosus* and *A. bahia*, and post exposure feeding rate for *N. arenaceodentata*.

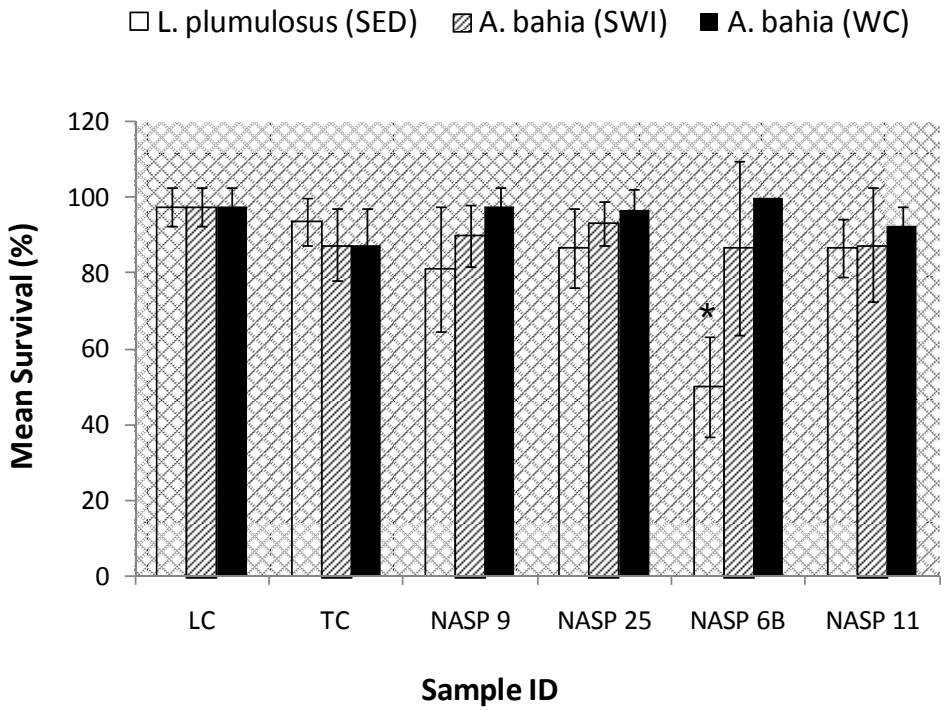

Figure 9-15. Results from 96 h in situ toxicity tests at NAS Pensacola. LC=laboratory control; TC=travel control.

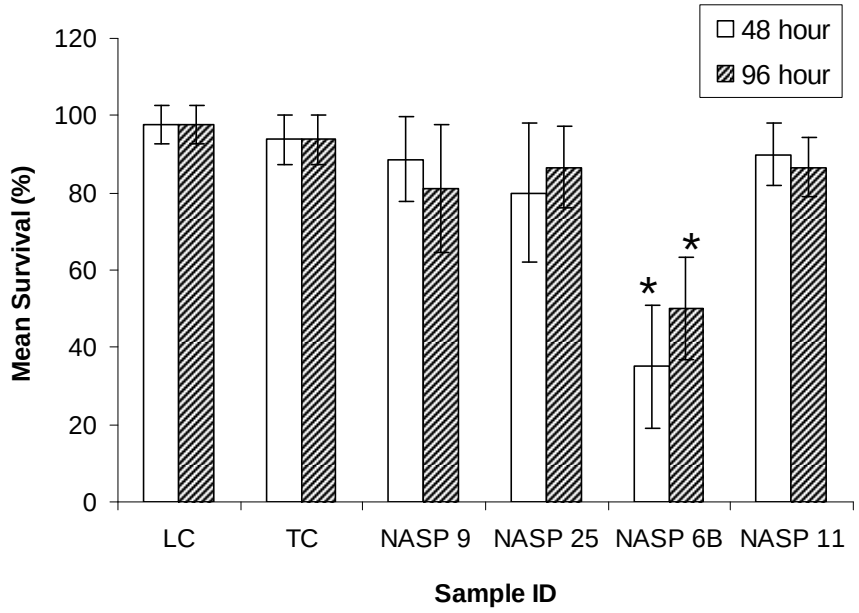

Figure 9-16. Comparison of 48 and 96 h *in situ* toxicity tests at NAS Pensacola using *L. plumulosus*. LC=laboratory control; TC=travel control. * indicates statistically lower than LC and TCs.

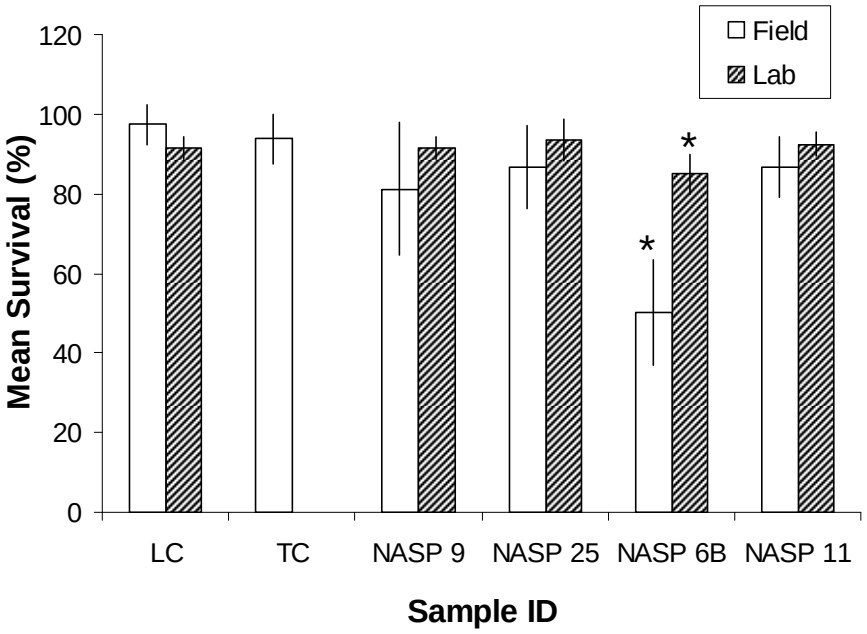

Figure 9-17. Comparison of 48 h in situ and laboratory surficial sediment toxicity tests with *L. plumulosus* at NAS Pensacola.

NAS Pensacola Water Quality. Continuously measured water quality results from the *in situ* exposures at NAS Pensacola are summarized in Table 9-5. All parameters were within those tolerated by the test species and were very similar to the lab exposure conditions. Oxidation-reduction potential at stations NASP6B and NASP25 suggested conditions were reducing.

NAS Pensacola Bulk Sediment Chemistry. Bulk sediment concentrations from the four focal stations are shown in Table 9-6. Among the three sites evaluated in this project, chemical concentrations in bulk sediment were overall highest at NASP, with stations NASP6B and NASP25 exhibiting the highest concentrations. Metals including Cd, Cr, and Pb were above sediment quality guidelines using the effect range median (ERM) criterion, as were g-BHC (lindane) for both stations. NASP 6B also exceeded the ERM for total DDTs. Both stations exceeded the effect range low (ERL) for total PAHs.

NAS Pensacola Pore Water and SWI Chemistry. Measured pore water chemical concentrations were largely below detection limits at the four focal stations for divalent metals (RL = 0.2-300 µg/L), individual PAHs (RL= 0.1-0.21 µg/L), and pesticides (RL=0.021 ug/L). Chromium (76 µg/L) and nickel (8.7 µg/L) were measured in station 6B pore water. Very low concentrations of total DDT and its conjugates (expressed as sum) were measured at stations 6B (0.073 µg/L) and NASP 9 (0.008 µg/L). Dissolved organic carbon concentrations in the pore water were relatively high at all stations (6B=15.8 mg/L; 9= 11.3 mg/L; NASP11 = 15.9 mg/L; NASP 25; 5.0 mg/L). No chemical contaminants were detected in any of the SWI samples.

NAS Pensacola Bioaccumulation. *In-situ* and laboratory PAH body residues for *L. plumulosus* and *M. mercenaria* are shown in Table 9-10. *In-situ* exposures were conducted for 4-days, and lab exposures were conducted for 4 and 28-days (*L. plumulosus*), with survival and lipid content of the amphipods being substantially reduced in the latter. For the *in-situ* exposures, PAHs were only detected in the *L. plumulosus* tissues, while all samples for *M. mercenaria* were below detection limits. For *L. plumulosus* tissues, PAHs were detected only at stations NASP6B and NASP25 during *in situ* exposures, but at background concentrations at the other two stations during laboratory exposures. Laboratory exposed amphipods had higher body residues (factor of 2 to 3) than *in situ* exposed amphipods. Lab results showed similar trends in the 4-day exposures with very low levels in *M. mercenaria*, and higher levels in *L. plumulosus* at NASP6B and NASP25.

Table 9-10. Total PAH (EPA 16 priority) tissue concentrations for laboratory and in situ bioaccumulation exposures with *Leptocheirus plumulosus* (marine amphipod) and *Mercenaria mercenaria* (hard clam) at NAS Pensacola.

Species	Exposure Duration	Location Unit	Lab (µg/kg ww)		Lab (µg/kg lipid)		*In Situ* (µg/kg ww)		*In Situ* (µg/kg lipid)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
L. plumulosus	4 days	Control	98.7	28.6	6763	1959	-	-		
		6B	350.2	129.6	19392	7177	155.3	*	10641	*
		9	105.4	33.6	7601	2419	0	*	0	*
		11	25.3	9.4	1710	636	0	*	0	*
		25	477.7	306.5	29599	18991	159.1	*	10902	*
	28 days	Control	*ND*	*ND*	*ND*	*ND*	-	-	-	-
		6B	*ND*	*ND*	*ND*	*ND*	-	-	-	-
		9	*32.3*	*34.1*	*3,585*	*3,792*	-	-	-	-
		11	*0.0*	*0*	*0.0*	*0*	-	-	-	-
		25	*147.5*	*8.4*	*492*	*27.9*	-	-	-	-
M. mercenaria	4 days	Control	0.0	-	0	-	<32.4	-	<32.4	-
		6B	17.3	15.3	1040	921	<32.4	-	<32.4	-
		9	36.9	43.5	2878	3399	<32.4	-	<32.4	-
		11	0.0	-	0	-	<32.4	-	<32.4	-
		25	0.0	-	0	-	<32.4	-	<32.4	-
	28 days	Control	17.833	15.4	1783	1544	-	-	-	-
		6B	9	16.1	715	1239	-	-	-	-
		9	<32.4	-	<32.4	-	-	-	-	-
		11	<32.4	-	<32.4	-	-	-	-	-
		25	<32.4	-	<32.4	-	-	-	-	-

ND=no data due to poor survival of L. plumulosus at day 28
< values indicate values were below method detection limit and reporting limits.
Dash indicates measurements not made.
*Indicates no standard deviation calculated due to need to combine replicates
Italics indicate that 28 day exposed *L. plumulosus* had poor survival and variable lipid content, therefore, data are suspect.

NAS Pensacola SPME. Figure 9-18 shows a plot of the measured tissue concentrations for five PAHs (Pyrene, B[a]a, B[b]F, B[k]F, and B[a]P) versus organic carbon-normalized solid-phase concentrations and PDMS-derived pore water concentrations, along with linear fits to the data. The concentrations of other PAHs were below detection limits. For simplicity, the five PAHs were combined for analysis, but the researchers at the University of Texas are currently examining potentially more appropriate ways to compare these data, due to differences in expected partitioning based on K_{ow}. SPME-derived pore water concentrations were highly correlated (r^2>0.977) with tissue concentrations determined in both lab and *in situ* amphipod exposures. Pore water and tissue concentrations were highest at NASP 25, followed by NASP6B>NASP9>NASP11.

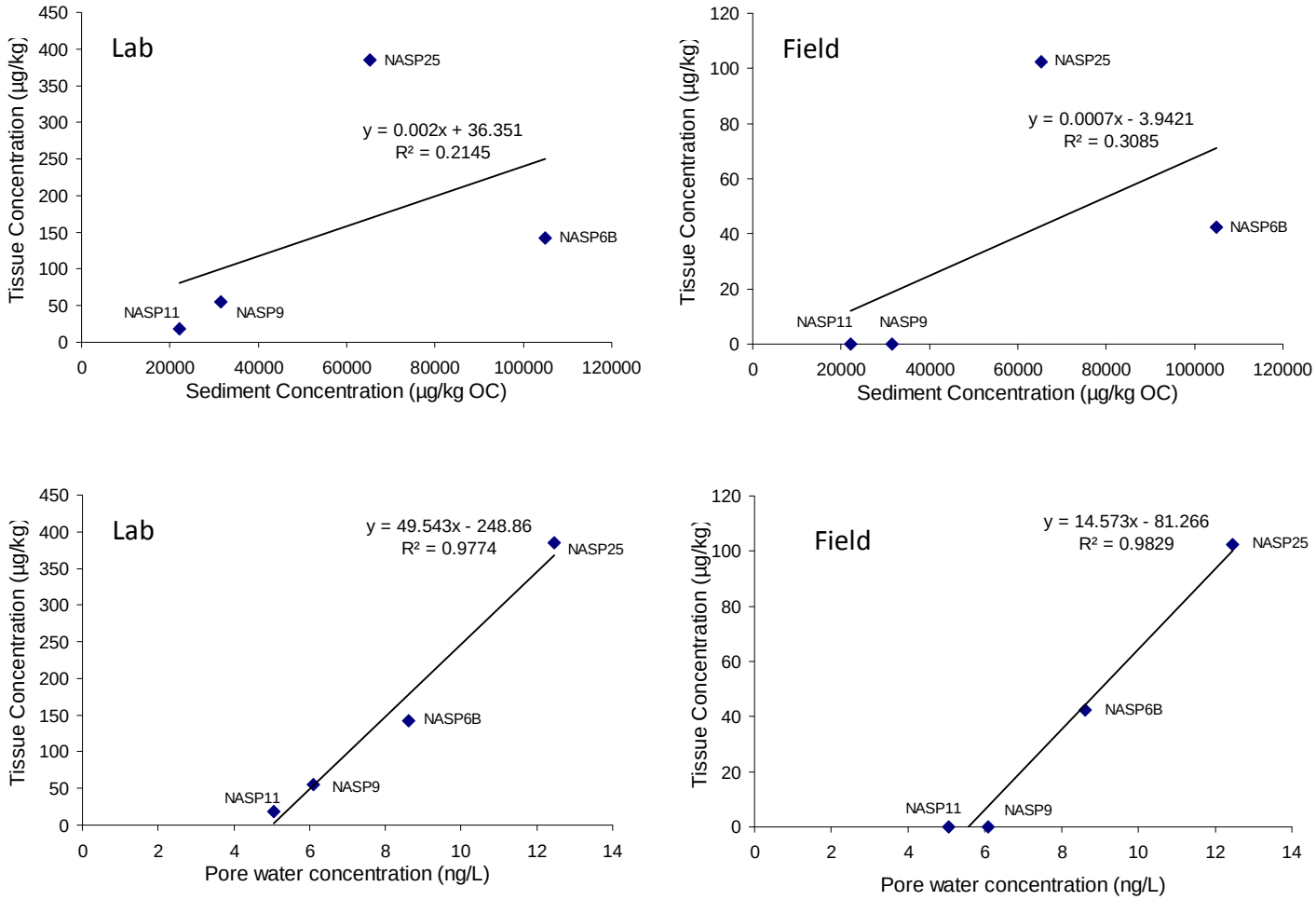

Figure 9-18. Uptake of PAHs from 96 h exposures with the burrowing amphipod *Leptocheirus plumulosus* at Naval Air Station Pensacola in both lab (left) and in situ (right). Top figures represent comparisons of organisms uptake with organic carbon normalized sediment concentrations. Bottom figures represent organism uptake relative to SPME-derive pore water concentrations. For simplicity, each data point represents the sum of pyrene, B[a]a, B[b]f, B[k], and B[a]P from the four stations.

NAS Pensacola DGT. Effective concentrations in sediments are shown (by depth) in Figure 9-19. Values were highest for zinc, followed by nickel and lead. Concentrations of copper and cadmium were similar when detected; however, copper was not detected at station(s) NASP25, and was only detected in the deeper sediments of the remaining sites. On average, metal concentrations were lowest at station NASP9 (the reference station), while copper, zinc and cadmium were highest at NASP11, and nickel and lead highest at NASP6B (Table 9-11).

Table 9-11. Concentrations (µg/l) of metals measured using diffusive gradient thin films (DGT) probes, Bayou Grande, FL, November 2008. Reported concentrations from DGT probes are averaged across the top 5 cm of the sediment.

Site	Cu	Zn	Ni	Pb	Cd
NASP9	0.028	1.509	0.305	0.067	0.049
NASP11	0.072	12.78	0.625	0.064	0.060
NASP25	0	5.033	0.894	0.155	0.039
NASP6B	0.028	4.942	0.666	0.095	0.039
NASP6X	0.013	1.629	1.627	0.096	0.081
NASP6Y	0	1.113	0.594	0.008	0.001
NASP6Z	0	0.853	0.601	0.052	0.009

In contrast to the San Diego results, metal concentrations within these sediments tended to increase with increasing depth for all metals (Figure 9-19). Concentrations were generally similar (within a factor of two) relative to NBSD, but were more than an order of magnitude lower for copper (when detected).

Coefficients of determination (r^2) for NASP bulk sediment concentration and C_E were as follows: Cu (0.544); Zn (0.020); Pb (0.879); Cd (0.720); and Ni (0.799), however, Cu, Zn, and Cd comparisons were all inversely related.

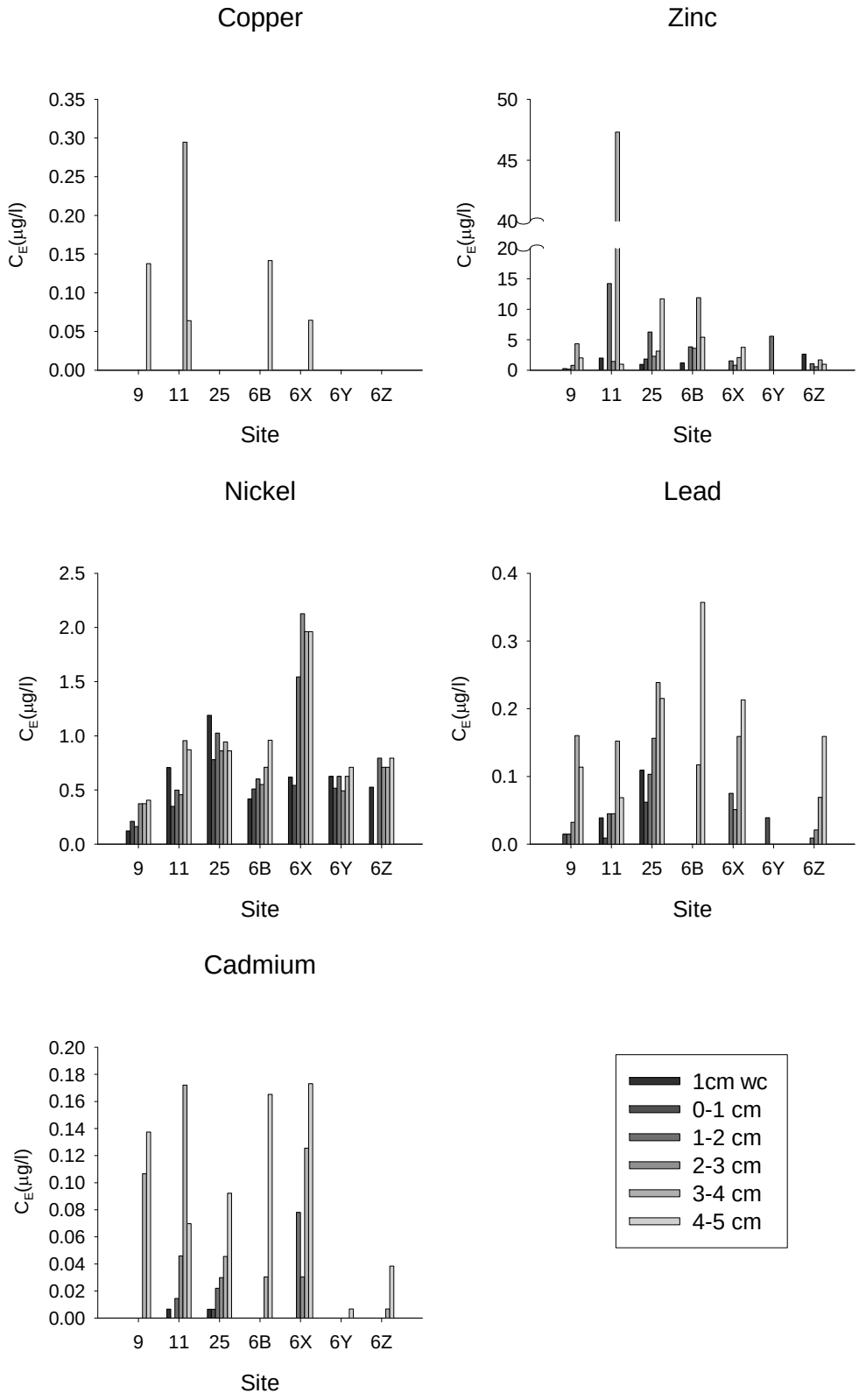

Figure 9-19. DGT results for NAS Pensacola.

9.4.3. Chollas Creek Results

Toxicity. For most stations at Chollas Creek, amphipod (*E. estuarius*) survival was high (>80%) after 48 h of exposure, with at least some reduction observed with increasing proximity to or contact with the sediment. Survival in SED exposures was significantly lower ($p<0.05$) than the laboratory controls at the three Chollas Creek stations, but not at the reference site (CP2243). Survival in the SWI exposures was somewhat lower than in WC exposures, but generally not significantly. Survival in SED and SWI exposures was most markedly reduced at station C14 (24 ± 28% survival; Figure 9-20). Polychaete (*N. arenaceodentata*) post exposure feeding rate from SED exposures was also lowest at C14 (Figure 9-21), but station C10 also resulted in statistically lower feeding rates ($P<0.05$), while the reference station (CP2243) did not.

Water quality. Overlying water quality in SED exposures was for the most part acceptable, but DO concentration at station C14 declined to levels approaching 0 mg/L for short periods of time in the evening hours prior to increasing again in the early morning hours (Table 9-5; Figure 9-22). It should be noted that some parameters were not recorded at this site due to an error during the setup of the probes preventing data capture. However, discrete water quality samples taken from chambers prior to recovery showed otherwise normal water quality.

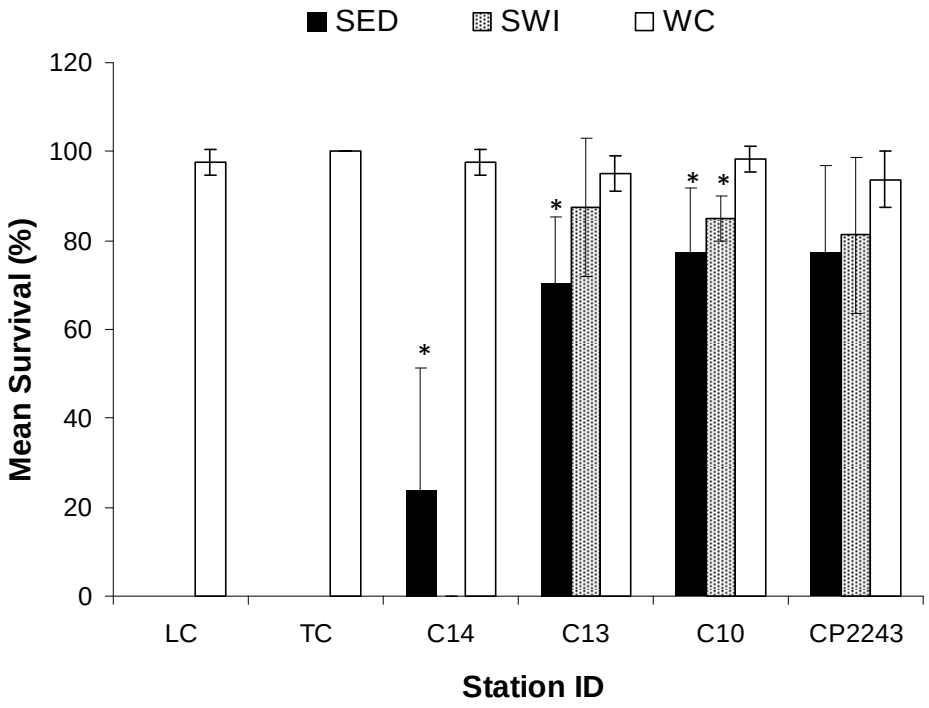

Figure 9-20. *In situ* survival (± 1 standard deviation) of *E. estuarius* following 48 h exposure at Chollas Creek, San Diego, CA. Contaminant exposure source was investigated with concurrent exposure in three compartments (SED=surficial sediment; SWI=sediment-water interface; WC=water column). LC=laboratory control, TC=travel control. * indicates statistical significance ($p<0.05$) relative to laboratory control.

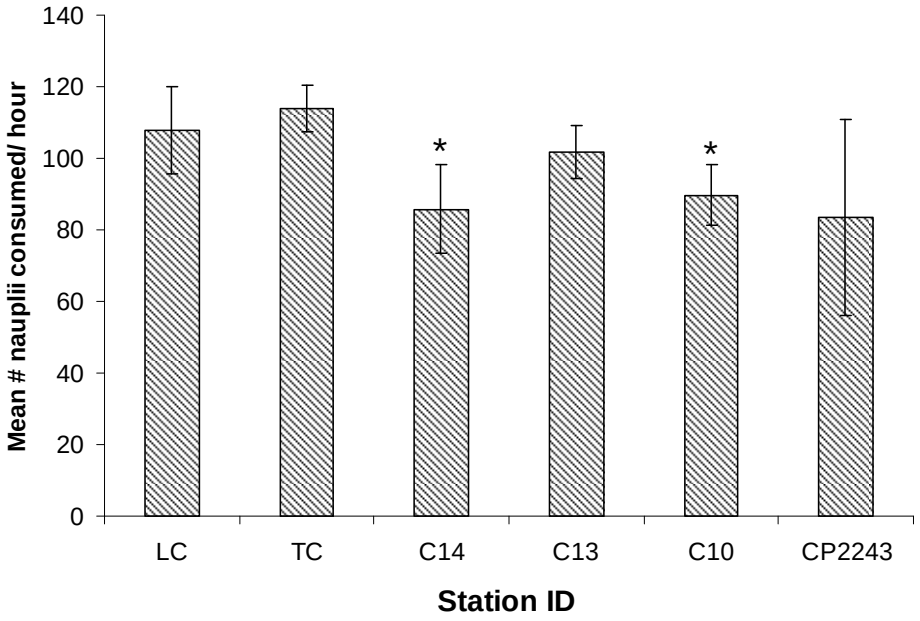

Figure 9-21. Post *in situ* exposure feeding rate (± 1 standard deviation) of *N. arenaceodentata* following 48 h exposure at Chollas Creek, San Diego, CA. LC=laboratory control, TC=travel control. * indicates statistical significance ($p<0.05$) relative to laboratory control.

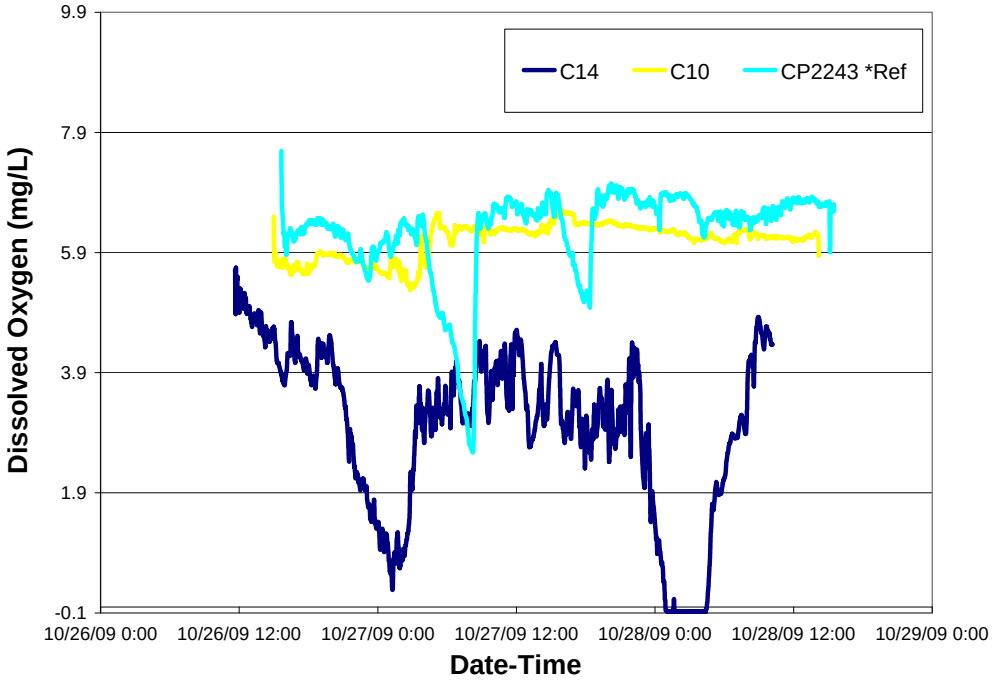

Figure 9-22. Continuously measured dissolved oxygen concentrations inside representative sediment exposure chambers during in situ Chollas Creek deployment.

Organism uptake. PAH uptake was elevated, by factors of up to three and four, relative to the reference site, at the three Chollas Creek test stations for both *M. senhousia* (Table 9-12) and *E. estuarius* (Table 9-13), respectively (Figure 9-23). PAH body burdens followed the expected historical contamination gradient, with the highest concentrations generally observed at stations C13 and C14.

Table 9-12. PAH body residues (ug/kg wet wt.) for *M. senhousia* following 48 h sediment exposure in situ at Chollas Creek site PAH body residues (ug/kg wet wt.) for M. *senhousia* following 48 h sediment exposure in situ at Chollas Creek site. N=3 replicates. Dash indicates not calculable because only one replicate resulted in a value above the method detection limit, or values were below reporting limit. < indicates value was below both method detection limit and reporting limit. Italics indicate values were above method detection limit, but below reporting limit.

	M.senhousia									
	Unexposed		C14		C13		C10		CP2243	
Analyte	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Acenaphthene	<8.3	-	*3.9*	*0.8*	<7.4	-	*3.8*	-	<8.1	-
Acenaphthylene	<8.3	-	*3.3*	*0.4*	<7.4	-	*3.2*	-	<8.1	-
Anthracene	11.2	-	*7.1*	*3.8*	<7.4	-	*3.1*	-	3.5	-
Benzo (a) anthracene	<8.3	-	14.8	5.6	10.8	0.6	11.0	1.5	6.8	1.7
Benzo (a) pyrene	<8.3	-	8.6	3.8	6.3	0.3	9.6	1.7	4.8	1.3
Benzo (b) fluoranthene	<8.3	-	20.9	10.4	16.1	6.8	19.3	3.3	7.9	1.0
Benzo (g,h,i) perylene	<8.3	-	16.0	10.8	7.0	2.6	6.0	0.5	4.0	1.1
Benzo (k) fluoranthene	<8.3	-	14.1	6.9	9.9	0.2	18.1	6.8	6.4	1.3
Chrysene	<8.3	-	17.2	2.2	15.5	1.1	12.0	1.1	6.9	1.5
Dibenz (a,h) anthracene	<8.3	-	13.1	8.4	5.7	1.5	*5.0*	*0.5*	3.9	0.4
Fluoranthene	<8.3	-	35.0	0.9	32.4	1.3	18.4	0.1	11.7	2.8
Fluorene	<8.3	-	*5.1*	-	3.3	0.3	4.1	0.1	3.2	-
Indeno (1,2,3-cd) pyrene	<8.3	-	9.6	4.7	6.0	1.5	7.5	1.0	4.2	-
Naphthalene	<8.3	-	10.6	2.8	8.5	0.4	8.4	2.6	8.0	1.7
Phenanthrene	<8.3	-	10.9	0.1	7.6	0.0	9.3	0.5	5.0	1.5
Pyrene	*7.6*	-	40.6	0.2	49.7	2.3	27.0	1.4	10.2	0.1
TPAH	18.8	-	231	62	179	19	166	21	87	14

Table 9-13. PAH body residues (ug/kg wet wt.) for *E. estuarius* following 48 h sediment exposure *in situ* at Chollas Creek. N=2 replicates (each replicate is a composite of survivors of four individual replicate exposure chambers). Dash indicates not calculable because only one replicate resulted in a value above the method detection limit, or values were below reporting limit. < indicates value was below both method detection limit and reporting limit. Italics indicate values were above method detection limit, but below reporting limit.

	E. estuarius									
	Unexposed		C14		C13		C10		CP2243	
Analyte	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Acenaphthene	<11.8	-	*4.2*	-	*7.19*	-	<10.1	-	<13.2	-
Acenaphthylene	<11.8	-	<10.5	-	<10.5	-	<10.1	-	<13.2	-
Anthracene	<11.8	-	<10.5	-	<10.5	-	<10.1	-	<13.2	-
Benzo (a) anthracene	<11.8	-	*6.7*	-	12.6	0.2	*6.5*	-	*5.8*	-
Benzo (a) pyrene	<11.8	-	<10.5	-	*6.1*	*1.5*	*6.5*	-	<13.2	-
Benzo (b) fluoranthene	<11.8	-	10.9	-	15.5	0.4	17.4	-	*9.6*	*2.3*
Benzo (g,h,i) perylene	<11.8	-	<10.5	-	*4.4*	-	*4.4*	-	<13.2	-
Benzo (k) fluoranthene	<11.8	-	*5.9*	-	*9.0*	*2.0*	11.3	-	*6.4*	*0.1*
Chrysene	<11.8	-	11.7	-	20.5	3.5	*8.5*	-	*4.7*	-
Dibenz (a,h) anthracene	<11.8	-	<10.5	-	<10.5	-	<10.1	-	<13.2	-
Fluoranthene	<11.8	-	51.2	13.0	68.2	16.8	25.0	-	*11.1*	-
Fluorene	<11.8	-	*5.03*	-	*6.8*	-	<10.1	-	<13.2	-
Indeno (1,2,3-cd) pyrene	<11.8	-	<10.5	-	*4.4*	-	*4.4*	-	<13.2	-
Naphthalene	<11.8	-	*8.6*	*4.4*	*6.8*	-	*8.1*	-	*8.1*	*1.7*
Phenanthrene	15.6	-	27.0	7.9	12.6	9.4	29.5	-	19.1	0.8
Pyrene	*5.68*	-	53.7	10.0	174.0	-	39.6	-	*10.6*	*1.5*
TPAH	21.28	-	185	35.4	348	55.1	161	-	75	6.5

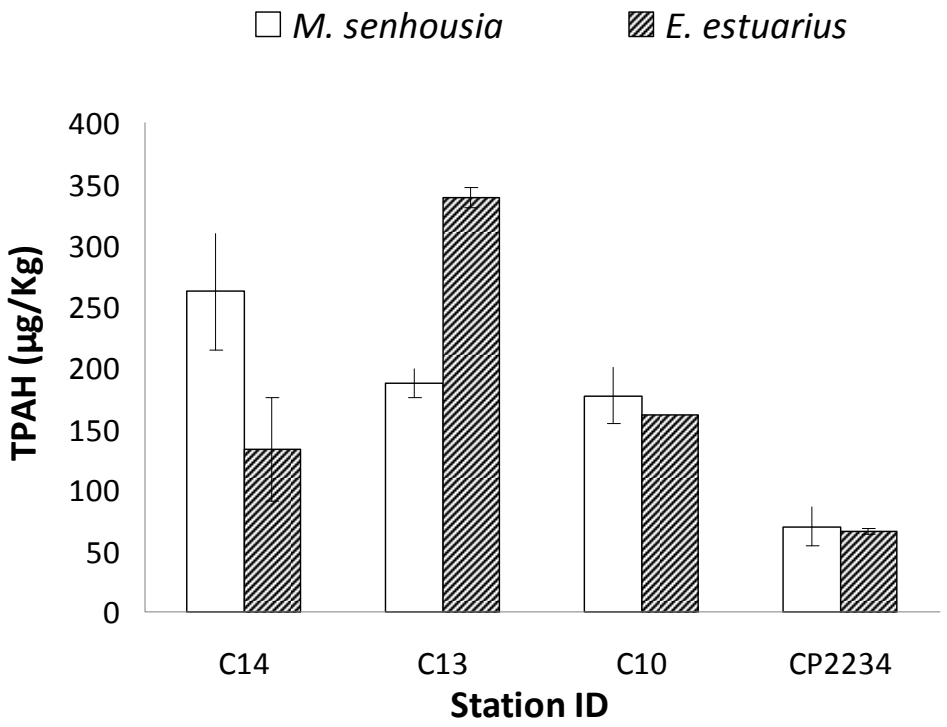

Figure 9-23. Comparison of infaunal mussel (*M. senhousia*) and burrowing amphipod (*E. estuarius*) total PAH uptake following 48 h exposure in situ at Chollas Creek site. N= 2 composites of 3-4 individual chambers each. CP2234 is the reference location.

Pesticide body residues were measured for *M. senhousia* only. All organophosphate and chlorinated pesticides were below the method detection limits, except DDTs. Total DDTs were slightly elevated at stations C14 and C10 (Table 9-14), but at background levels for C13 and the reference location (CP2243).

Metal body residue data for *M. senhousia* and *E. estuarius* are shown in Table 9-15 and Table 9-16, respectively. For *M. senhousia*, Cd, Cr, Cu, Pb, Ag, and Zn were elevated (typically by about a factor of two or less) at station C14, relative to the reference site (CP2243). Body residues at stations C13 and C10 were also somewhat elevated relative to the reference site, especially for Pb and Cd, but were overall lower than concentrations measured at station C14. Metal body burdens for *E. estuarius* did not show a clear trend in most cases.

Table 9-14. Pesticide body residues (mg/kg wet wt.) for *M. senhousia* following 48 h sediment exposure in situ. N=2 replicates (each replicate is a composite of survivors of four individual replicate exposure chambers). Dash indicates not calculable because only one replicate resulted in a value above the method detection limit, or values were below reporting limit. < indicates value was below both method detection limit and reporting limit. Italics indicate values were above method detection limit, but below reporting limit.

	M.senhousia									
	Unexposed		C14		C13		C10		CP2243	
Analyte	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
4,4´-DDD	3.14	-	2.62	-	ND	-	ND	-	ND	-
4,4'-DDD [2C]	ND	-	3.07	-	3.17	1.1	4.54	0.2	4.00	1.0
4,4´-DDE	<2.5	-	ND	-	ND	-	<2.5	-	ND	-
4,4'-DDE [2C]	ND	-	*0.722*	*0.2*	*0.747*	*0.3*	*0.746*	-	*0.786*	*0.2*
4,4´-DDT	2.50	-	4.31	-	ND	-	3.69	-	ND	-
4,4'-DDT [2C]	ND	-	2.87	-	*2.36*	*0.8*	3.32	-	3.17	0.8
Total DDTs	5.64	-	13.6	-	6.28	-	12.3	-	7.96	-
Aldrin	<10	-	<10	-	<10	-	<10	-	<10	-
alpha-BHC	<10	-	<10	-	<10	-	<10	-	<10	-
alpha-Chlordane	<10	-	<10	-	<10	-	<10	-	<10	-
beta-BHC	<10	-	<10	-	<10	-	<10	-	<10	-
delta-BHC	<10	-	<10	-	<10	-	<10	-	<10	-
Dieldrin	<10	-	<10	-	<10	-	<10	-	<10	-
Endosulfan I	<10	-	<10	-	<10	-	<10	-	<10	-
Endosulfan II	<10	-	<10	-	<10	-	<10	-	<10	-
Endosulfan sulfate	<10	-	<10	-	<10	-	<10	-	<10	-
Endrin	<10	-	<10	-	<10	-	<10	-	<10	-
Endrin aldehyde	<10	-	<10	-	<10	-	<10	-	<10	-
Endrin ketone	<10	-	<10	-	<10	-	<10	-	<10	-
gamma-BHC (Lindane)	<10	-	<10	-	<10	-	<10	-	<10	-
gamma-Chlordane	<10	-	<10	-	<10	-	<10	-	<10	-
Heptachlor	<10	-	<10	-	<10	-	<10	-	<10	-
Heptachlor epoxide	<10	-	<10	-	<10	-	<10	-	<10	-
Methoxychlor	<10	-	<10	-	<10	-	<10	-	<10	-

Table 9-15. Metal body residues (mg/kg wet wt.) for *M. senhousia* following 48 h sediment exposure in situ. N=2 replicates (each replicate is a composite of survivors of four individual replicate exposure chambers). < indicates value was below both method detection limit and reporting limit. Italics indicate values were above method detection limit, but below reporting limit. Dash indicates not calculable.

	M.senhousia									
	Unexposed		C14		C13		C10		CP2243	
Analyte	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
4,4´-DDD	3.14	-	2.62	-	ND	-	ND	-	ND	-
4,4'-DDD [2C]	ND	-	3.07	-	3.17	1.1	4.54	0.2	4.00	1.0
4,4´-DDE	<2.5	-	ND	-	ND	-	<2.5	-	ND	-
4,4'-DDE [2C]	ND	-	*0.722*	*0.2*	*0.747*	*0.3*	*0.746*	-	*0.786*	*0.2*
4,4´-DDT	2.50	-	4.31	-	ND	-	3.69	-	ND	-
4,4'-DDT [2C]	ND	-	2.87	-	*2.36*	*0.8*	3.32	-	3.17	0.8
Total DDTs	5.64	-	13.6	-	6.28	-	12.3	-	7.96	-
Aldrin	<10	-	<10	-	<10	-	<10	-	<10	-
alpha-BHC	<10	-	<10	-	<10	-	<10	-	<10	-
alpha-Chlordane	<10	-	<10	-	<10	-	<10	-	<10	-
beta-BHC	<10	-	<10	-	<10	-	<10	-	<10	-
delta-BHC	<10	-	<10	-	<10	-	<10	-	<10	-
Dieldrin	<10	-	<10	-	<10	-	<10	-	<10	-
Endosulfan I	<10	-	<10	-	<10	-	<10	-	<10	-
Endosulfan II	<10	-	<10	-	<10	-	<10	-	<10	-
Endosulfan sulfate	<10	-	<10	-	<10	-	<10	-	<10	-
Endrin	<10	-	<10	-	<10	-	<10	-	<10	-
Endrin aldehyde	<10	-	<10	-	<10	-	<10	-	<10	-
Endrin ketone	<10	-	<10	-	<10	-	<10	-	<10	-
gamma-BHC (Lindane)	<10	-	<10	-	<10	-	<10	-	<10	-
gamma-Chlordane	<10	-	<10	-	<10	-	<10	-	<10	-
Heptachlor	<10	-	<10	-	<10	-	<10	-	<10	-
Heptachlor epoxide	<10	-	<10	-	<10	-	<10	-	<10	-
Methoxychlor	<10	-	<10	-	<10	-	<10	-	<10	-

Table 9-16. Metal body residues (mg/kg wet wt.) for *E. estuarius* following 48 h sediment exposure in situ. N=2 replicates (each replicate is a composite of survivors of four individual replicate exposure chambers). < indicates value was below both method detection limit and reporting limit. Italics indicate values were above method detection limit, but below reporting limit. Dash indicates not calculable.

		E. estuarius									
		Unexposed*		C14*		C13		C10*		2243	
Analyte	RDL	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Aluminum	0.18	8.17	-	63.4	-	94.9	3.96	70.3	-	68.3	5.52
Antimony	0.045	<0.049	-	<0.360	-	<0.334	-	0.452	-	<0.613	-
Arsenic	0.045	1.59	-	1.51	-	1.59	0.170	1.37	-	2.04	0.375
Barium	0.045	4.09	-	5.64	-	5.22	0.233	5.05	-	7.79	2.26
Beryllium	0.045	<0.049	-	<0.360	-	<0.334	-	<0.625	-	<0.613	-
Cadmium	0.045	0.061	-	<0.360	-	0.233	-	<0.625	-	<0.613	-
Calcium	0.902	11400	-	11000	-	11150	636	9650	-	13250	1626
Chromium	0.045	0.120	-	<0.360	-	0.349	0.071	0.324	-	<0.613	-
Cobalt	0.045	0.223	-	<0.360	-	0.228	-	<0.625	-	<0.613	-
Copper	0.045	19.7	-	22.5	-	22.9	3.11	20.9	-	29.9	6.43
Iron	0.18	116	-	189	-	226	26.9	187	-	215	34.6
Lead	0.045	0.354	-	<0.360	-	2.10	2.33	0.307	-	<0.613	-
Magnesium	0.18	1330	-	1650	-	1585	21.2	1560	-	1965	247
Manganese	0.045	1.38	-	2.13	-	2.34	0.403	3.51	-	5.60	1.99
Molybdenum	0.045	0.137	-	<0.360	-	<0.334	-	<0.625	-	<0.613	-
Nickel	0.045	0.886	-	1.18	-	1.12	0.170	0.97	-	1.70	0.382
Potassium	0.902	1660	-	1780	-	1670	99.0	1540	-	2210	311
Selenium	0.045	0.346	-	<0.360	-	0.365	0.060	0.305	-	<0.613	-
Silver	0.045	0.081	-	<0.360	-	<0.334	-	<0.625	-	<0.613	-
Thallium	0.045	<0.049	-	<0.360	-	<0.334	-	<0.625	-	<0.613	-
Tin	0.045	2.16	-	11.3	-	8.55	2.62	8.32	-	26.7	11.1
Vanadium	0.045	0.071	-	<0.360	-	0.329	0.012	<0.625	-	<0.613	-
Zinc	0.045	9.63	-	11.8	-	12.9	2.12	11.5	-	18.8	5.73

Passive samplers –SPME. The *in situ* SPME housing described by Lampert et al. (in prep) was slightly modified for this event. The top 3 cm was analyzed independently of other sediment depths, and likely represents the most relevant exposure zone for the surficial sediment dwelling invertebrates used in this study. The processing of these data are still in progress due to some concerns about handling the SPMEs during transit.

Bulk chemistry. Bulk sediment contaminant concentrations are summarized in Table 9-6. Total PAH were below ERLs, while total PCBs, total DDTs, and some divalent metals were above ERLs, but lower than ERMs. In general, station C14 exhibited lower bulk contaminant levels than C10 and C13. Station C14 had a particularly low silt/clay and TOC contents relative to the other stations, and consisted of a large amount of twig and leaf matter.

9.5. Discussion

9.5.1. NBSD Discussion

Toxicity. Overall the toxicity results suggest potentially elevated risk at the station closest to the quay wall (NS21), followed by stations NS24, NS22, and essentially no risk at the reference site (CP2243). The

high survival rates of *E. estuarius*, *N. arenaceodentata*, and *A. bahia* at the reference station (CP2243) and reduction in survival at historically contaminated stations show that incorporation of these commonly used laboratory test species are appropriate for use as *in situ* indicators of toxicological risk. To our knowledge, use of these organisms in the field are limited to only a few studies (e.g. Anderson et al. 2004 [*E. estuarius*]; Clark et al. 1987 [*A. bahia*]), with no known previous *in situ* toxicity testing with *N. arenaceodentata.*

The elevated toxicity observed for *E. estuarius* and *N. arenaceodentata* assays in sediment exposures at station NS21, with an absence of effects at any test station for *A. bahia* in water column exposures points to the sediment as the exposure source. The positive correlation between 2-day *in situ* and 10-day laboratory exposures was interesting, but not expected. *In situ* toxicity tests do not necessarily result in the same responses, nor do they tend to reliably result in greater or lesser effects, but are determined by site-specific factors (Tucker and Burton 1999, Kater et al. 2001, Ringwood and Keppler 2002, Anderson et al. 2004, Burton et al. 2005). It is interesting to note, however, that similar responses were observed in this study even though the *in situ* exposure was only two days. Burton et al. (2005) also reported the tendency to observe responses more rapidly in the field in freshwater *in situ* toxicity tests. Some degree of sediment heterogeneity and other factors such as possible predatory-related effects may be the cause for the relatively higher variability observed in the *in situ* exposures with *E. estuarius.* This was also noted by Anderson et al. (2004), who suggested increasing replication in field exposures, which should be considered in future studies.

The post exposure feeding rate assay with *N. arenaceodentata* was recently developed and demonstrated in aqueous exposures as part of SERDP #ER-1550 (Rosen and Miller, in press; Section 7.0). This assay provides a new simple, short-term, sub-lethal response test that can provide the realism offered by *in situ* exposures while maintaining laboratory control during the effects assessment phase. Initial field use of the assay appears promising, based on high recoveries of the test organisms from the field for feeding rate assessment, and a range of responses observed. At NBSD, the feeding rate assay corresponded well with the amphipod assay in terms of ranking of the most toxic stations, with stations NS21 and NS24 resulted in more reduced feeding relative to NS22 and CP2243.

The highly variable results observed from the *M. galloprovincialis* embryo-larval development exposures *in situ* appeared to be primarily a result of the presence of high quantities of sediment particles and detrital material that agglomerated after entering the small mesh chambers, making microscopic examination of the larvae extremely difficult. Normally developed larvae were observed at all stations, but the inability to account for all larvae in the exposure chamber or at least differentiate between abnormal larvae and other material in the chamber made assessment difficult. Water quality was also negatively impacted in some of the *in situ* SWI exposures with the mussel embryos, likely due to clogging of the very fine mesh (as low as 20 µm). Improvements in the exposure design for bivalve embryogenesis exposures *in situ* are currently being investigated, as this endpoint has high relevance and sensitivity, and represents an *in situ* version of the laboratory based SWI assay commonly employed in sediment risk assessments (Anderson et al. 1996). We have recently found that sand dollars and sea urchin embryos, which are nearly as sensitive to metals as mussel embryos, may be more amenable to exposure in situ (unpublished data). High recoveries of normally developed larvae in the concurrent laboratory-based SWI exposures that used intact core samples and mussel embryos, however, suggested that contaminant flux from the sediments was not an exposure route of concern at NBSD.

Organism uptake. Total PAH uptake in *M. senhousia* followed the same gradient as that observed for the toxicity results (NS21>NS24>NS22>CP2243). The highest concentration (NS21 = 98 µg/kg), however, was well below what would be considered to result in PAH-induced effects by non-polar narcosis (McCarty and Mackay 1993), which weren't observed with *M. senhousia.* However, the data provide another line of evidence towards characterizing the relative sediment quality among the four test stations at NBSD. Total PCB concentrations were similar in magnitude between *N. arenaceodentata* and *M.*

senhousia, suggesting either species are appropriate as *in situ* bioaccumulation organisms. Although total PCB concentrations were slightly higher than the reference station, differences were not large. This is likely due in part to the lower than expected PCB contamination (based on bulk concentrations) at the site. The relatively high PCB residues in *N. arenaceodentata* after only two days of exposure attest to the utility of short-term bioaccumulation testing for hot spot detection. These findings are consistent with *in situ* exposures with freshwater oligochaetes that showed high rates of PCB uptake over the first 48 h of exposure (Burton et al. 2005), suggesting that this approach is sufficient for delineating hot spots of contamination for hydrophobic organic contaminants.

SPME. PDMS-derived pore water PAH concentrations were better correlated to *M. senhousia* tissue concentrations than organic carbon solid-phase concentrations and those from pore water derived by centrifugation. This is a significant result as sediment quality benchmarks (e.g. Long et al. 1995) are often based on the solid-phase concentrations, which appear to be a weaker indicator of bioaccumulation potential than pore water concentrations. Lampert et al. (in prep) provide extensive detail on the mimetic capability of the *in situ* PDMS sampler as it relates to this study. The authors describe an examination of the relationship between predicted and measured tissue concentrations, and conclude that pore water concentrations (as derived by the samplers) predict bioaccumulation. However, pore water concentrations under-predicted tissue concentrations to some extent, which could be explained by overestimateion of the lipid-water partiion coefficient by K_{ow}, elimination effects, organism stress, and insufficient time for the organisms to reach equilibrium conditions. Other sources of variability in the data included measured pore water concentrations, variability in fiber geometry, variability in tissue concentrations, and variability in lipid content. Based on these sources of variability, their models based solely on K_{ow} and pore water concentration explain the majority of the observed variation in the data.

DGTs. The *in situ* deployed DGTs successfully characterized labile metals in surficial sediments at NBSD, generally corresponding with centrifugation-derived bulk pore water concentrations. Effective concentration (C_E) of a given metal is similar to the concentration of that metal in bulk porewater, provided the supply of the metal is rapid (Zhang et al. 2001). In addition, C_E automatically accounts for all sediment properties, including pH and total and dissolved organic carbon (Zhang et al. 2001). This combination of factors potentially explains the relatively high discrepancy between C_E and bulk porewater metal concentration for Pb, which is the most tightly bound of all the divalent metals to both organic carbon and AVS within sediments.

No distinct spatial patterns were evident within the sediment profiles, although there did appear to be an overall trend of decreasing nickel concentrations with depth at all sites, and decreasing copper concentrations at all sites except for NS21, the site closest to the quay wall (Figure 9-9). Concentrations of Pb and Zn also increased with depth at site NS21; however, concentrations of Cd and Ni decreased with increasing depth. Other authors have found metal concentrations in marine sediments to increase with increasing sediment depth (e.g. Boothman et al. 2001), and have hypothesized that shallower sediments lose metals as a result of bioturbation, physical turbulence, and diffusive processes. However, these observations were made on sediments spiked with metals, and at a coarser spatial resolution than what was measured in this study. It is possible that the upper layers of these loosely attached, fine particulate sediments redistribute themselves via physical and biological turbation with enough frequency that the metal concentrations in the top 5 cm of sediments at these sites are largely the result of vertical spatial heterogeneity.

Although Ni and Zn C_E correlated well with bulk sediment concentrations, no positive correlation was observed for the other metals, further illustrating that sediment quality guidelines based on bulk concentrations do not sufficiently predict bioavailability, and the importance of tools such as DGTs for assessment of the bioavailable fraction. Although the C_E's measured in this study were unlikely to explain any of the observed effects at NBSD and do not consistently show specific trends in terms of overall magnitude for all metals at any one station, overall bioavailability of metals was consistently

greater at the site relative to the reference station. It is suspected that evaluation at sites with a larger gradient in metals contaminant levels and sediment quality characteristics (i.e. grain size, organic carbon content) would have provided more distinct differences.

NBSD Conclusions

This first full scale deployment of the SEA Rings was successfully executed and involved a pairing of *in situ* rapid toxicity and bioaccumulation testing species/endpoints relevant to the San Diego region with experimental *in situ* SPME devices (in collaboration with the University of Texas), and continuous water quality sensing. A contamination gradient observed historically and as part of this study was observed with relatively highest concentrations of PAHs and metals nearest the quay wall, while lowest concentrations were observed at the reference site. This corresponded with *in situ,* and in some cases laboratory, toxicity test results with amphipod survival and polychaetes post exposure feeding rate. Similar toxicity responses were observed in *in situ* and laboratory exposures with amphipods, however, the former showed that such a response could be observed in only two days. Lack of responses in water column deployed mysids suggested they are a robust species for *in situ* use, but appeared to eliminate concern about effects associated with that compartment, while embryogenesis exposure methods appear to need refinement to eliminate small mesh water quality effects and issues associated with differentiating between abnormal larvae and sediment particles during microscopic examination. The utility of the PDMS SPME devices was clear based on positive correlations between uptake by *in situ* deployed bivalves and the pore water concentration as determined by the fibers, while comparisons of uptake with solid-phase concentrations were less predictive. Likewise, DGT samplers showed effective concentrations that overall did not correspond with bulk sediment concentrations, illustrating their utility, while providing a vertical gradient of labile metals and a direct means for assessment of potential toxicity associated with metals.

9.5.2. NAS Pensacola Discussion

Of the three sites (NBSD, NASP, and Chollas Creek) examined in this project, the site at NAS Pensacola involved the most comprehensive demonstration of the SEAP. Integration of hydrological assessment tools, on site pore water toxicity screening, *in situ* and laboratory bioassays, *in situ* uptake from organisms and passive samplers, and chemical concentrations from bulk and interstitial water phases were performed in a rapid, sequential manner in a single trip to the field site. The Trident Sensor survey guided placement of SEA Rings and the UltraSeep to help characterize ecological risk at four focus stations, providing a weight-of evidence approach for reduced uncertainty through more realistic and integrated measures that, in combination with traditional assessment methods, helped assess the level of risk at the site.

9.5.2.1. NAS Pensacola Groundwater Discharge Zone Assessment

In general, the groundwater discharge zone evaluation revealed shoreline areas with evidence of groundwater discharge (Trident survey) which was quantified at one location (NASP25), via an UltraSeep deployment, with a mean rate of about 1 cm/day. Daily fluctuations in tide have been shown to lead to appreciable volumes of groundwater extraction into the overlying water via the process of tidal pumping (Moore 1996). Although the more soluble groundwater contaminants such as the chlorinated volatile organic compounds (VOCs) can be attenuated as they near the sediment surface (Duncan et al. 2000), seepage measurements and pore water sampling at coastal sites have indicated concentrations that could impact biological communities at the groundwater–surface water interface (Chadwick et al. 1999). In this study, however, pore water and discharge water chemical characterization indicated that there was no VOC discharge associated with the groundwater discharge, with the possible exception of trace levels of naphthalene and 1,2,4-Trichlorobenzene in pore water at NASP5.

9.5.2.2. In Situ Integrated Assessment

Toxicity vs. body residues. High control survival and lack of response at the reference site suggest that the three species used for toxicity at NAS Pensacola are amenable for estuarine *in situ* toxicity testing. To our knowledge, neither *L. plumulosus* nor *N. arenaceodentata* have been used previously in *in situ* bioassays. *A. bahia,* however, has been used successfully during *in situ* assessment of effects associated with pesticide spray applications (Clark et al. 1987).

The effort to assess exposure source at this site indicated that the sediment was the most likely exposure source, as no effects were observed in SWI or WC *A. bahia* exposures. As with the NBSD study, amphipod and polychaete tests responded similarly, this time only exhibiting effects at one station (NASP 6B), with only the former being statistically significant. Unlike NBSD, *in situ* and laboratory responses differed, with greater mortality associated with the *in situ* exposure. Differences between *in situ* and laboratory responses have been reported frequently when both types of studies are conducted concurrently (Tucker and Burton 1999, Kater et al. 2001, Ringwood and Keppler 2002, Anderson et al. 2004, Burton et al. 2005), and is a testament to the utility of inclusion of *in situ* toxicity tests in the risk assessment process as an often important additional line of evidence.

Although pesticides (total DDTs and Lindane), total PAHs, and several divalent metals were present above various sediment quality benchmarks at the two southernmost stations (NASP 6B and 25), very low bioavailability and toxicity of these contaminants was observed based on the variety of synoptically collected *in situ* measurements. The lack of any noteworthy contaminant levels in the UltraSeep discharge, pore water, or SWI water samples, and the absence of major water quality changes observed during the *in situ* exposures, suggests that there is no indication that groundwater discharge contained contaminants of concern, nor mobilized existing surficial sediment contaminants. This appeared to be corroborated by generally low uptake and SPME-derived pore water concentrations.

Uptake of PAHs was elevated in *L. plumulosus* deployed at both NASP25 and 6B. However, these concentrations do not alone explain the observed toxicity at 6B based on critical body residue (CBR) theory (McCarty and Mackay 1993). Non-polar organic contaminants that act by narcosis (i.e. PAHs) require a body residue in the range of 2 to 8 mmol/kg to produce acute mortality in invertebrates and fish (McCarty and Mackay, 1993), but sum PAH molar concentrations for NASP 6B were only 0.0016 mmol kg, three orders magnitude lower. In addition, body residues at the nearby station NASP 25 were about 50% higher (0.0021 mmol kg), where no acute toxicity was observed.

It is possible that differences between lab and field toxicity results at NASP 6B could be associated with UV-light induced photo-toxicity of PAHs that might have occurred in the field, as has been suggested or observed by others (Sasson-Brickson and Burton 1991, Ireland et al. 1996, Pelletier et al. 1997). The bottom of the SEA Rings were positioned at an average depth of only 0.6 m at NASP 6B, and were as low as 0.1 m during low tide (Table 9-5). This could be verified in future studies at this site by comparing responses in UV-light exposed chambers alongside chambers that are shaded. Ireland et al. (1996), for example, reported an absence of toxicity from shaded *Ceriodaphnia dubia* chambers, but substantial mortality from chambers exposed to direct sunlight (all other parameters being equal) in 48-hour *in situ* exposures in PAH-contaminated sediments.

Chlorinated pesticides may also have contributed to *L. plumulosus* mortality at 6B, as total DDTs and g-BHC (Lindane) exceeded sediment quality benchmarks (Long et al. 1995; MacDonald et al. 1996). Concentrations, however, were not analyzed in the tissues, and bioavailability of these chemicals was expected to be low based on the high organic carbon content of the sediments. In addition, bulk sediment concentrations were two and one order(s) of magnitude below those that would be expected to induce acute lethality in laboratory spiked-sediment exposures with *L. plumulosus* (DDTs; Lotufo et al. 2001) and the amphipod *Gammarus locasta* (g-BHC; Costa et al. 1998).

The sediments at the NASP site were on the whole organically rich (TOC content up to 7.63%; NASP 25) relative to the San Diego based sites. The fact that sediment organic carbon plays such a large role in the bioavailability and toxicity of contaminated sediments, with the contaminated sediments preferentially partitioning to the organic carbon fraction, likely explains relatively low pore water concentrations, and apparently low bioavailability, at the two stations where some contaminants were elevated in the bulk chemistry, may be reflective of relatively high total organic carbon in those samples.

Water quality. Water quality parameters measured in representative *in situ* sediment chambers (sensor positioned at sediment-water interface in sediment chamber) indicate that water quality was sufficient at station NASP 6B (and all stations) to maintain organism health. Interestingly, salinity, pH, and ORP, however, were noticeably lower at 6B when compared to the other three *in situ* locations. Ammonia was not suspected to contribute to toxicity at station NASP 6B based on total concentrations (9 mg/L) measured in discrete pore water samples, below those expected to cause toxicity to *L. plumulosus.*

Differences in organism uptake. In this study, only PAHs were analyzed in tissues of laboratory and *in situ* deployed test organisms. The substantially higher body burdens (based on total internal PAH concentration) for *L. plumulosus* relative to *M. mercenaria* is likely a function of the small size of the former, having faster uptake kinetics, as well as more direct opportunity for exposure. *L. plumulosus* is a sediment burrowing deposit feeding amphipod, while *M. mercenaria* is a filter feeder that likely ingested relatively little sediment, trapping particles in the overlying water instead. Furthermore, *M. mercenaria* may have reduced exposure for parts of the exposure via valve closure.

DGT. Although bulk metal concentrations were generally an order of magnitude greater at stations NASP6B and NASP25, effective concentrations were highest at NASP11 for three of the five metals. Although positive correlations were observed for nickel and lead, the other metals were either weakly or inversely correlated. Therefore, as observed at NBSD, bulk metal concentrations were not a good surrogate for the labile fraction.

Interestingly, copper was not detected by DGT at NASP 25, yet it possessed the highest bulk sediment concentration (230 mg/kg), which approached the copper ERM (277 mg/kg). The apparent lack of copper bioavailability at station NSP25 is likely associated with factors such as high silt/clay content, total organic carbon content, and documented reducing conditions at that station, based on the continuous water quality sensing data.

In contrast to the San Diego results, metal concentrations within these sediments tended to increase with increasing depth for all metals (Figure 9-19). This is more consistent with previous observations (e.g. Boothman et al. 2001), and may be the result of coarser sediments, a more stable hydrological environment, or some combination of these and other factors.

The overall lack of concordance among replicate DGTs deployed at station 6B is interesting to note. As with the other stations, the NASP 6B DGT was deployed with the SEA ring suspended from a slightly modified test organism exposure chamber (Figure 8-3, Section 8.0). The replicate DGTs (6X, 6Y, and 6Z), however were separately inserted into nearby sediments. It is unclear as to whether the differences in concentrations were the result of how they were deployed or were simply the result of local spatial heterogeneity.

9.5.2.3. NASP Integrated In-Situ Sediment Assessment Conclusions

The integrated *in-situ* sediment assessment at the NAS Pensacola site generally reflects areas of low to moderate chemical loading in the bulk sediment with limited bioavailability, uptake or response. While bulk concentrations in sediment sometimes exceeded screening benchmarks (i.e. total DDTs, g-BHC, total PAHs, divalent metals), other measures of exposure including porewater, discharge water, interface water and passive samplers largely indicate a lack of mobility and bioavailability. This is supported by the

lack or limited uptake of PAHs in tissues of exposed organisms, and the general absence of toxicity in either laboratory or *in situ* exposed organisms. The disparity between the lab and field toxicity data at the southernmost station show that results from lab studies do not necessarily explain effects that may be observed in the field, highlighting the relevance of inclusion of *in situ* studies. The shallow depth of the southernmost station (NASP6B) may have resulted in toxicity due to UV-photoactivation of the accumulated PAHs.

In situ assessment at this site provided the opportunity to improve characterization of exposure and response at a site with ground water-surface water interactions, using tools that could simultaneously characterize the GSI exposure magnitude as well as the potential for effects across several tidal cycles, which would have been impossible in the laboratory with grab samples. In general, chemical analysis of pore water, sediment-water interface, and flow weighted discharge samples, along with organism bioaccumulation, passive samplers, and toxicity tests provided multiple lines of evidence that suggest that sites upstream of the wetland (where GSI were of historical concern) do not appear to correspond with ecological risk downstream, and also indicates that bulk sediment concentrations would not be a reasonable indicator of ecological risk at the site. Similar to this study, Greenberg et al. (2002) used hydrologic and chemistry data from nested minipiezometers to improve the interpretation of exposure–effects relationships along a river contaminated with chlorobenzenes.

9.5.3. Chollas Creek Discussion

Chollas Creek Toxicity. Similar to the NBSD study, Chollas Creek sediment quality appears to have improved over recent years. The compartmentalization of amphipods into WC, SWI, and SED exposure chambers successfully demonstrated an absence of water column induced effects. Water column recoveries were ≥94% at all stations, indicating that the SEA Rings provided an appropriate means for successfully deploying and recovering standard benthic invertebrates traditionally used in laboratory toxicity tests from deep water, high traffic, marine systems such as areas near the mouth of Chollas Creek. The absence of water column toxicity is noteworthy due to the mouth of Chollas Creek being on the receiving end of a very industrialized urban watershed with numerous point and non-point discharges that historically demonstrate toxicity to both freshwater and marine invertebrates (Schiff et al. 2002).

The only station that exhibited a substantial reduction in *in situ* amphipod survival was station C14, where both SWI and SED exposed amphipods were highly impacted. In addition to consideration of the various measurements discussed below, it should be noted that reduced water quality and/or sediment physical characteristics could have played a role in the observed response at C14. Continuous measurements made by the water quality sensors revealed nightly precipitous declines (to as low as 0 mg/L) in D.O. concentration followed by sharp increases to acceptable levels by early morning (Figure 9-22, Figure 9-4). Sediment at this station was very organic in nature, consisting of large masses of detrital material including twigs and leaves. This, in combination with the shallow depth (1.5-2.5 m) and high residence time expected from this tributary of San Diego Bay (Chadwick et al. 2004) likely promoted ideal conditions for growth of benthic algae that could produce such diurnal D.O. fluctuations. The impacts on survival at C14 clearly illustrate the importance of continuous monitoring of physical parameters during *in situ* toxicity tests, as there is a high likelihood that grab water quality samples collected upon deployment and recovery (conducted during daylight hours) would not have captured the D.O. decline.

Contrary to *E. estuarius* survival, no statistically significant reduction of post exposure feeding rate by *N. arenaceodentata* was observed at any of the Chollas Creek stations. This difference could be due to differential sensitivity between the two tests to bioavailable contaminants, differences in exposure route (i.e. free burrower vs. tube builder), and/or differential sensitivity to stress by reduced D.O. concentration or other physiological stressors. At least one study has shown that *N. arenaceodentata* can survive prolonged (96 hour) exposure to D.O. concentrations as low as 1 mg/L (Dillon et al. 1993). It is also well established that marine polychaetes metabolize PAHs (Jørgensen et al. 2008), facilitated by cytochrome

P450 enzyme induction, which may result in transformation of bioaccumulated PAHs to more soluble, excretable products. Regardless of the lack of toxicity observed, this study suggests that the *N. areanaceodentata* feeding rate assay shows promise as an *in situ* assessment tool of contaminated sediment exposure and effects

Chollas Creek storm water discharges, which ultimately lead to the mouth of the creek and San Diego Bay, have been shown to be highly toxic, with toxicity identification evaluations (TIEs) pointing to organophosphate pesticides (such as diazinon and chlorpyrifos), and metals (copper and zinc) as causal agents (Schiff et al. 2002). Historical sediment assessments also exhibited toxicity to benthic invertebrates in laboratory studies, and showed bulk chemistry that exceeded sediment quality guidelines for PAHs, PCBs, metals, and chlorinated pesticides, including DDTs and chlordanes (SCCWRP and SSC San Diego 2002, Brown and Bay 2005). Subsequent preliminary sediment TIEs suggested that nonpolar organics were responsible for toxicity to *E. estuarius* in laboratory solid-phase toxicity tests (Bay and Greenstein 2002). A temporal study of toxicity and sediment chemistry at Chollas Creek demonstrated high variability of effects and some contaminant levels over time (Brown and Bay 2005). In that study, survival of *E.estuarius* in 10-day lab tests ranged from 2-79% at station C14, illustrating the potential for variable response over time. Bulk sediment chemistry, however, varied by only a factor of two or less for PAHs and PCBs, with the former being at concentrations up to 5 times greater than those observed in this study.

Despite the previously elevated contamination observed at station C14, and observed toxicity at that station during the present study, synoptically collected bulk sediment concentrations suggest that the contamination at Chollas Creek has improved. The spatial and temporal variability in chemical and toxicological results previously observed at the site, however, should be considered.

Bioaccumulation and biomimetics. For *M. senhousia, in situ* PAH body residues followed the expected historical contamination gradient among the four stations at Chollas Creek, with the highest TPAH concentration (231 ug/Kg) at C14 (Table 9-12, Figure 9-23). Although the highest body residue for *E. estuarius* was observed at station C13, the overall magnitude of uptake between the two species was similar. The observed residues from this study are an order of magnitude lower than those historically reported using the clam *Macoma nasuta* in 28-d exposures (SCCWRP and SSC San Diego 2002). In neither case, however, would the toxicity observed at station C14 be explained by PAH uptake alone. Critical body residue theory for PAHs suggest that narcosis-related toxicity is expected to occur at tissue concentrations ranging from 2 to 8 mmol/kg (McCarty and Mackey 1993; Kane Driscoll et al. 1997), which is approximately three orders of magnitude greater than those measured in the current study.

For both species, body residues were above both unexposed and reference site body residues, suggesting that 48 h was a sufficient period of time to observe differences among the sites, a criterion required for use of this rapid assessment approach as a means of identifying hot spots of contamination. Others also report rapid and significant uptake of non-polar organic contaminants in *in situ* exposures of short duration with small benthic invertebrates (Greenberg et al. 2002, Burton et al. 2005).

The concurrent SPME deployment at Chollas Creek suggested very low PAH pore water concentrations overall. These data are still being reviewed, however, due to concerns about the way the SPMEs were handled prior to analysis, and are not included so as not improperly interpret these data.

Chollas Creek Conclusions. This study showed that short-term, deep water *in situ* exposure and effects assessments adjacent to heavily populated, high traffic estuarine environments are feasible and informative in the assessment of ecological risk. The importance of continuous water quality measurements was particularly apparent in proper interpretation of toxicity data obtained at this site, which likely would have been missed with grab samples. Although lack of control of physico-chemical parameters is often considered one of the limitations of *in situ* toxicity testing, the ability to reduce

uncertainty with interpretation of field data is improved with measures such as these. The multiple lines of evidence based on bulk chemistry, toxicity, organism uptake, and use of PDMS passive samplers (SPMEs) suggests that conditions at the site have overall improved from reports just a few years ago, and present relatively low ecological risk to the benthic community.

It should be noted, however, that this study examined only three stations near the creek mouth and is by no means exhaustive. In addition, both spatial and temporal variability of toxicity, bulk chemistry, and benthic community structure have been observed at the site, even over short time periods (Bay and Brown 2005). Upstream flows to the creek mouth, particularly during storms, can potentially introduce pulses of increased exposure. Boat traffic in the area may also routinely disrupt surficial sediments, thereby altering the vertical stratification of constituents and redox gradients.

The overall lack of chemical contamination in surficial sediments relative to historical concentrations may be due to a combination of reduced inputs and natural attenuation. A number of regionally organized restoration efforts and recent bans on, and documented reductions of, toxic chemicals such as diazinon in discharges to Chollas Creek (Watanabe et al. 2008) may already be providing relief downstream of the 30 mile long creek. A recent TMDL to reduce diazinon and metals loading into the watershed will hopefully result in further improvements.

The decreased use of organophosphate pesticides in the Chollas Creek watershed over recent years has raised concern about the potential increase in new emerging replacement chemicals such as pyrethroid pesticides which are relatively toxic to amphipods such as *E. estuarius* (Anderson et al. 2008, 2010). In this study, which focused on the mouth of Chollas Creek, little evidence of elevated pesticides, including pyrethroids, was observed based on bulk chemical analysis (Figure 9-5).

9.6. Overall Conclusions

The integration of various endpoints and measures was useful in characterizing the sites investigated. Toxicity, bioaccumulation, bulk chemistry, and bioavailability as deemed by pore water concentrations derived from uptake by passive samplers followed the expected gradient at NBSD, and suggested hydrophobic organics (i.e. PAHs) might be important stressors, while bulk metals and DGT concentrations appeared to be of less concern. At NAS Pensacola, similar results were observed. However, the Trident and UltraSeep were used to evaluate the potential for groundwater-surface water interactions to be contributing to historically reported toxic effects at the southern end of the water body. Although groundwater was discharging into the surficial sediments, analysis of flow-weighted samples of the discharge revealed little to no chemical contamination associated with the infiltrating groundwater. Bulk chemistry, toxicity, and bioaccumulation, however, pointed to possible PAH-associated toxicity, which could have been exacerbated by UV photoinduced toxicity, explaining the difference between *in situ* and laboratory data for the shallow site. The importance of continuous water quality sensing was very clear at the Chollas Creek site, where diurnal drops in dissolved oxygen may have contributed to amphipod toxicity. That site, however, showed overall lower bulk chemical concentrations and toxicity than previously reported. This could be associated with recent restoration efforts upstream and reduced inputs of organophosphate pesticides, but the potential for temporal and spatial variability of results was noted.

9.7. Acknowledgements

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10.0 GIS WEIGHT OF EVIDENCE/WEIGHTED LOGISTIC REGRESSION ANALYSES OF SAN DIEGO BAY

10.1. Abstract

This section describes the final phase of the SEAP approach, utilizing existing and collected physical, chemical, indigenous biota, toxicity, and bioaccumulation data to provide a weight-of-evidence based evaluation of the data. This then allows for a ranking of sites based on ecological risk and identifies potential dominant stressors that are adversely impacting biota. Biological, sediment chemistry, and physical habitat data were sampled throughout the San Diego Harbor in the vicinity of the SPAWAR San Diego Harbor Naval Station. Field data resulting from a 2008 sampling for the Strategic Environmental Research and Development Program (SERDP) was supplemented with data collected from previous monitoring studies throughout the harbor study area and analyzed utilizing a geographic information systems (GIS)-based weights-of-evidence and weighted logistic regression (WOE/WLR) approach. In addition to more traditional assessment and data analysis approaches, the output generated by this spatial analysis provides relative rankings of stressor influence and ecological risk based on spatial patterns of environmental conditions and sampling probability. A WOE/WLR analysis was applied on the harbor study area to delineate potential stressor-response relationships between various biological responses (benthic infauna characteristics and *in situ* survival for amphipods), and seven environmental variables (depth, total organic carbon, % fines, total PCBs, priority pollutant PAHs, general metals, and cumulative pesticides). The main outcomes of this study are summarized as follows:

- Based on the spatial analysis results, the ecological risk of sediment contaminants within the naval station dock area targeted during the 2008 SERDP field program was determined to be relatively low for the biological responses evaluated in this study. Biological condition is likely more dependent on other environmental factors within the dock area.
- Most ecological risk predicted in the spatial analysis was concentrated in the Chollas Creek and Paleta Creek portions of the study area, highlighting the significance of these sites as the primary locations of ecological risk relative to the rest of the harbor study area.
- Pesticide exposure (represented as cumulative pesticide exposure) generally contributed the greatest increase in ecological risk in areas where biological impact was predicted, pointing to this stressor source as remediation priority where exposure occurs.
- Spatial analysis performed at both a larger harbor study area extent and a smaller inner harbor study area extent provided a more comprehensive ecological risk assessment.

Overall, the application of the spatial analysis approach was technically successful in delineating screening-level stressor hypotheses for use in site management, and showed potential for the application of this approach to similar study areas in the future.

10.2. Introduction

The goal of the Strategic Environmental Research and Development Program (SERDP) Project ER-1550 is the development of effective assessment tools and approaches for management of sites with historically contaminated sediment. Contaminant exposure and effects measures were sampled in the San Diego harbor in the vicinity of the SPAWAR San Diego Harbor Naval Station using a suite of laboratory, field physical, chemical, and toxicity sediment screening tools including Trident/UltraSeep System deployments. As a specific component of the project, field data generated during the course of the project, as well as supplemental archival data from previous monitoring programs in the corresponding harbor study area vicinity, was analyzed by a geographic information system (GIS) based analysis approach linking environmental conditions and biological effects. An exploratory application of a weight-of-evidence and weighted logistic regression (WOE/WLR, ArcSDM software for ArcGIS:

Sawatzky et al. 2004), previously applied to regional watershed assessment in an ecological risk assessment context (ex. Kapo et al. 2008), was extrapolated to the bay study area at two different spatial scales. A benefit of this spatial analysis approach applied to ecological risk assessment is the ability to estimate the relative influence of multiple stressors based on spatial associations between environmental conditions and biological response variables accounting for sampling probability and the geographic extent of a particular study area. Analysis model outputs provide stressor-response hypotheses and quantitative lines of evidence for assessing or developing remediation strategies and future monitoring studies. The use and integration of multiple lines of evidence in both the field and analysis components of the assessment yield a more comprehensive characterization of site condition and ecological risk.

In this study, field data (sediment chemistry and habitat, biological assessment, toxicity) collected in June 2008 in the San Diego harbor in the vicinity of the SPAWAR Naval Station was combined with archival data from previous monitoring studies within the general study area (Southern California Coastal Water Research Project: Bight 1998, Bight 2003, and Chollas and Paleta 2005 studies) in an extrapolated application of the WOE/WLR approach. The objectives of this study were 1) to generate stressor-response hypotheses (contaminants and/or other environmental conditions) relevant to ecological risk assessment for the study area to inform strategies for site managers and other stakeholders, and more generally, 2) to evaluate the potential and applicability of this type of spatial analysis approach to sediment assessments for sites such as this particular study area. One specific challenge to the second objective was the extrapolation of an approach applied previously to large-scale, relatively evenly-sampled watershed regions (ex. Kapo et al. 2008) to a significantly smaller-scale open-water study area with a sampling design heavily distributed throughout the inner harbor dock area where historical contamination was of most concern, and a smaller number of sample sites distributed in the large outer harbor area (Figure 10-1).

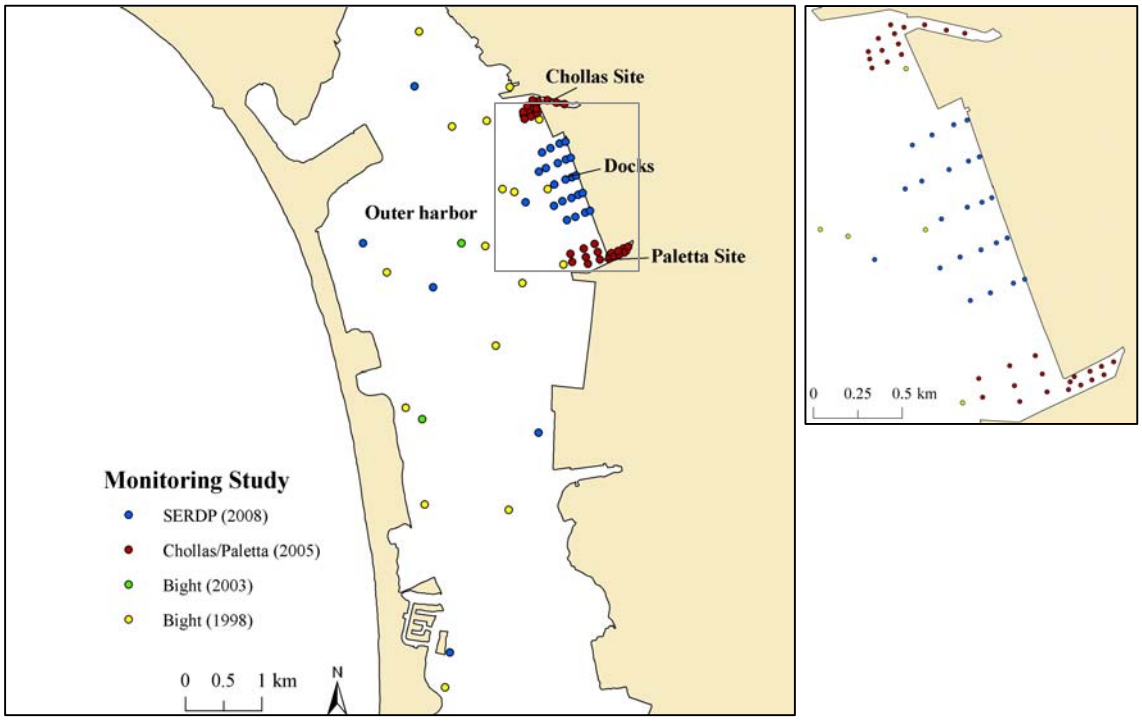

Figure 10-1. San Diego Harbor study area (vicinity of SPAWAR San Diego Harbor U.S. Naval Base), showing 87 total sample locations (points colored by each respective monitoring study, including the 28 locations monitored in the 2008 SERDP field sampling . The insert (right) provides a zoomed-in view of the inner

harbor, including the confluence of Chollas Creek, the naval station dock area, and the confluence of Paleta Creek. The boundary data source utilized was from Cal-Atlas, www.atlas.ca.gov.

This challenge was addressed in a number of ways. First, it was especially important to account for the influence of geographic sampling bias in the computation and analysis of stressor-response spatial patterns and relationships (detailed later). Additionally, the inclusion of archival data to the SERDP 2008 field data provided an improved spatial coverage of the harbor study area and increased sample size (reducing extrapolation uncertainty for unsampled locations), as well as better representation of the influence of inflowing waters (Chollas and Paleta Creeks) on environmental conditions in the harbor study area. Finally, the spatial analysis in this study was also performed at two spatial scales, 1) a general harbor study area encompassing all inner and outer harbor sampling locations, and 2) a smaller inner-harbor study area encompassing the naval station dock area and the confluences of Chollas and Paleta Creeks. The advantage of the larger study area was greater variability in environmental conditions for delineating stressor-response relationships, but this scale had increased uncertainty due to a lower sampling density (more extrapolation to unsampled locations). The smaller study area provided a more uniform and dense sampling distribution, but lacked the degree of variability in environmental conditions of the larger study area scale. Collectively, however, the use of two spatial scales provided a more comprehensive assessment of potential stressor-response relationships.

The primary goal of the GIS-based WOE/WLR approach (originally developed for minerals exploration, ex. Agterberg et al. 1993) as applied to ecological risk assessment (Kapo and Burton, 2006 and Kapo et al. 2008) is the development of screening-level stressor hypotheses based on statistical spatial associations between a biological training point dataset and the spatial patterns of one or more environmental variables. Generally, the approach computes spatial associations between stressor and response variables within the context of sampling probability (WOE), and integrates the spatial patterns of significant stressors to predict the spatial occurrence of a biological response across the study area (WLR). The specific components of the analysis are detailed later in the *Methodology*. Output from the analysis includes relative rankings of environmental variables in relation to their influence on the odds of the occurrence of a biological response, and an associated probability map that geographically illustrates cumulative stressor influence. The quantitative and visual components of the analysis provide useful information for the characterization and communication of ecological risk. While challenges in extrapolating the spatial analysis approach to the specific characteristics of the study area in this project presented some limitations and uncertainties (discussed in more detail later), it did not preclude a successful technical application of the methodology. Overall, this study is best interpreted as a quantitative screening-level ecological risk assessment, as well as a case study of method application demonstrating various strengths, potential, and areas for improvement in the analysis, design and data components of the project.

10.3. Methodology

Approach Overview

Various biological and environmental variables based on field sample data from the San Diego harbor study area were used to provide training point datasets and environmental gradients, respectively, for the delineation of potential stressor-response relationships. In addition to 28 sample locations from the SERDP 2008 sampling program, 59 additional sample data locations from three archival datasets (Southern California Coastal Watershed Research Project, http://sccwrp.com, Bight 1998, Bight 2003, and Chollas/Paleta 2005 [samples collected in 2001]) within the general harbor study area (Figure 10-1) were used as supplemental data to increase sampling density in the study area. For any sample locations that were identical between monitoring programs, only the most recent data was used. While supplementation with archival data introduced some uncertainty to the analysis as far as temporal correspondence between monitoring programs, it most likely reduced overall analysis uncertainty by

providing estimates of environmental conditions in areas of the harbor not sampled in the 2008 SERDP analysis that would otherwise need to be estimated. In the WOE/WLR approach used in this study, an initial data exploration using WOE analysis was performed to identify potential stressor variables by determining significant spatial associations between biological response (point data) and various environmental gradients (raster data), accounting for sampling probability and geographic sampling bias. Potential stressors identified by the WOE analysis were then utilized as predictor variables in a logistic regression model (WLR) to predict the relative probability (i.e. favorability) of the occurrence of a biological response across the study area, with model coefficients providing a relative ranking of individual predictor influence in the model. Collinearity of environmental variables was systematically evaluated throughout the WOE/WLR process. Additionally, the spatial analysis was performed at the scale of the larger harbor study area, and a smaller area extent encompassing the inner harbor (shown in detail shortly).

Biological Response and Environmental Variables

Sample data utilized for this study included benthic infauna characterization data, amphipod *in situ* toxicity results, and natural and anthropogenic environmental variables for the 87 sample locations in the San Diego Harbor study area. Variable selection was guided by biological and environmental variables that were consistently sampled between respective monitoring programs, as well as results from a previous benthic infauna analysis study on the 2008 SERDP data (Aquatic Bioassay and Consulting Laboratories, 2008). Biological response variables were selected and defined as discrete training point datasets, as required for WOE/WLR analysis, using the 25th percentile and >75th percentile values as thresholds to represent poor quality and high quality sites in this study. The use of percentiles in this study to represent biological condition results in the analysis representing ecological "risk" in the direct context of the harbor study area. Alternatively, other thresholds could be selected based on user-defined criteria (for example, a minimum acceptable level value of a biological response). Two benthic characterization variables, and *in situ* results for amphipod toxicity were evaluated in this study (Table 10-1). The biological data was represented as point feature data at the geographic locations of the sampling events (Figure 10-2).

Table 10-1. Biological response variables and training dataset definitions for spatial analysis.

Benthic Infauna Characterization	***25th centile (N)***	***>75th centile (N)***
Species richness	<= 25 count (22)	> 40 count (20)
Total abundance	<= 237 count (22)	> 951 count (20)
Toxicity	***25th centile (N)***	***>75th centile (N)***
Amphipod *in situ* survival	<= 73% (20)	> 89% (23)

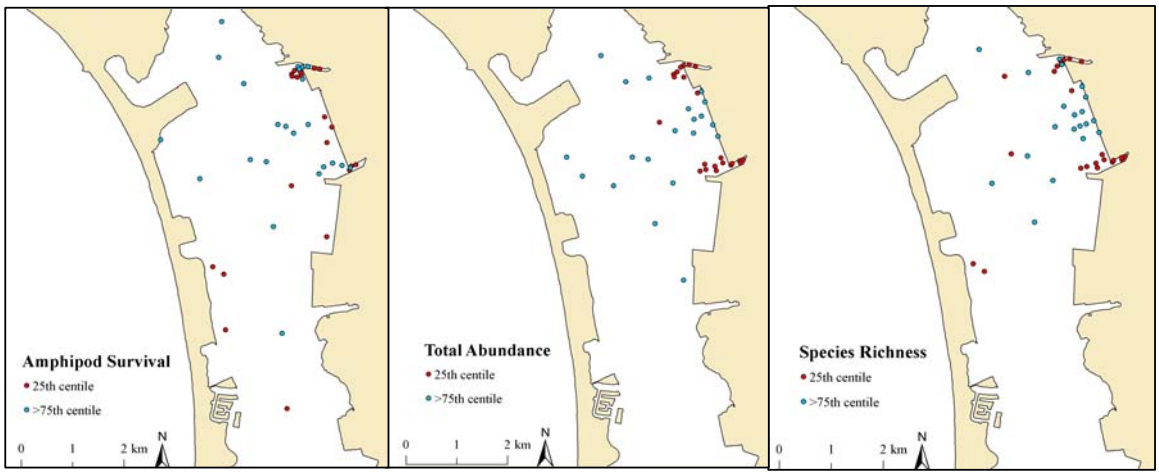

Figure 10-2. Locations of sites with low-quality (25th centile) and high-quality (75th centile) observations throughout the harbor study area for three biological response variables evaluated in this study: 1) amphipod survival, 2) total abundance (benthic infauna), and 3) species richness (benthic infauna). Thresholds for the percentile values are given in Table 10-1

Seven environmental variables representing sediment contaminants (4) and natural characteristics (3) were evaluated in this study (Table 10-2). Along with consistency of environmental variables between monitoring programs, correlations of principal component analysis (PCA) factors generated in the previous analysis of SERDP 2008 data (Aquatic Bioassay and Consulting Laboratories, 2008) with individual environmental variables was also used to select the variable that best represented general (cumulative) metals exposure. As a result, copper (Cu, mg/kg dry wt.) was selected to represent general exposure to metals because it was the variable consistently sampled between the various monitoring programs that had the highest correlation with the "General Metals" PCA factor derived in the previous benthic infauna analysis study (Aquatic Bioassay and Consulting Laboratories, 2008). Summations of sample measurements were used to represent organic contaminant and pesticide exposure (Table 10-2). Significant correlation was delineated amongst many environmental variables (Table 10-3). This was considered and incorporated systematically in the proceeding spatial analyses, described later. Additionally, the spatial data processing and classification procedures applied to the environmental variables, described shortly, also reduced correlative relationships observed in the raw data.

Table 10-2. Seven (7) environmental variables evaluated in this study.

Environmental Variables
Depth (m)
Fines (%)
Total organic carbon (TOC, %)
Metals (Cu, mg/kg dry wt.)
Total PCB (ug/kg dry wt.)
Priority pollutant PAH (PPAH, ug/kg dry wt)*
Total DDT (TDDT, ug/kg dry wt)**

** PPAH includes sum of: napthalene, acenapthylene, acenapthene, fluorene, anthracene, phenanthrene, fluroanthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3,-c,d)pyrene, dibenzo(a,h)anthracene, benzo(d,h,i)perylene*

*** TDDT includes sum of: 2,4'DDD, 4,4'DDD, 4,4'DDE, 4,4'DDT.*

Table 10-3. Pair-wise correlations (Spearman Rank) for environmental variables (N=87).

Variable Pair	Spearman Rank Correlation	*= p<0.05
PCB-TDDT	0.82	*
Cu-TOC	0.77	*
Cu-%Fines	0.73	*
PPAH-TDDT	0.72	*
%Fines-TOC	0.71	*
PCB-TPAH	0.59	*
PCB-TOC	0.57	*
PPAH-TOC	0.54	*
Cu-PCB	0.46	*
Cu-PPAH	0.42	*
Depth-%Fines	0.40	*
TDDT-TOC	0.40	*
Cu-Depth	0.31	*
Depth-TOC	0.25	*
Cu-TDDT	0.23	*
Depth-PPAH	0.22	*
%Fines-PCB	0.22	*
Depth-PCB	0.18	
Depth-TDDT	0.09	
Fines-PPAH	0.09	
Fines-TDDT	-0.08	

Study Area and Environmental Gradients

A general harbor study area of approximately 18 km^2 (Figure 10-3, left) was delineated in the San Diego Harbor based on the spatial extent of the inner and outer harbor sampling sites and land boundaries from the California Spatial Information Library (Cal-Atlas, www.atlas.ca.gov). Additionally, a smaller raster study area (0.8 km^2) including only the inner harbor was created (Figure 10-3, right). Raster were created for each study area with a resolution of 1 m^2. An even smaller study area comprising only the dock area of the inner harbor was also initially attempted, however that study area size did not provide an adequate spatial extent to yield analysis results. Spatial analysis at the larger harbor study area extent, and inner harbor extent (Figure 10-3) allowed adequate study area and data variability to perform a successful spatial analysis. Ordinary kriging interpolation (ArcGIS Geostatistical Analyst using default parameters) was performed on the environmental sample data points (N=87, Figure 10-1) to represent the spatial trends of each environmental variable across the harbor study area in raster format. This interpolation method was chosen to model the general gradient of environmental conditions across the study area, taking into account neighboring sample locations (5 nearest neighbors) in the estimation of variable values for each raster cell. The trade-off of interpolation relates to uncertainty at un-sampled portions of the study area, as the method chosen provides a representation of general spatial trends but can miss unknown anomalous patterns that may be present. To reduce interpolation uncertainty on the proceeding spatial analysis, each interpolated environmental variable raster was then reclassified into 5 general value classes (Jenk's Natural Breaks, ArcGIS) to generate a gradient of values across the study area for further analysis (Figure 10-4). Reclassification was done for both the large harbor study area, and the smaller inner harbor study area, respectively, to provide environmental gradients directly relevant to each study area.

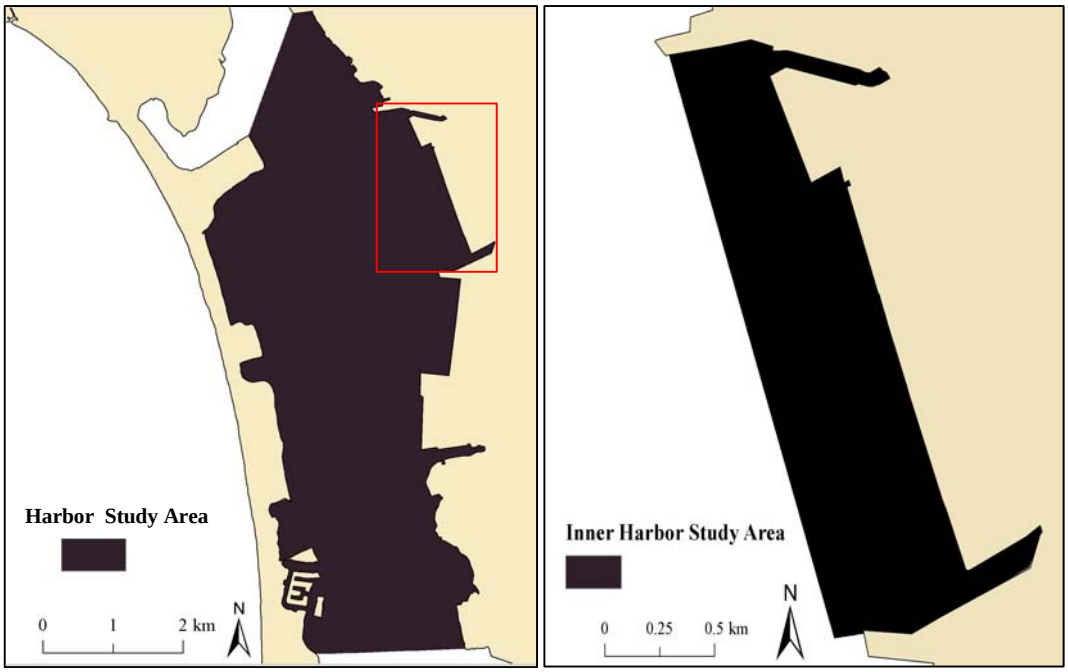

Figure 10-3. Raster study area shown for the general harbor study area (left), and the inner harbor study area (right). The location of the inner harbor study area relative to the larger harbor area is shown by the box outline.

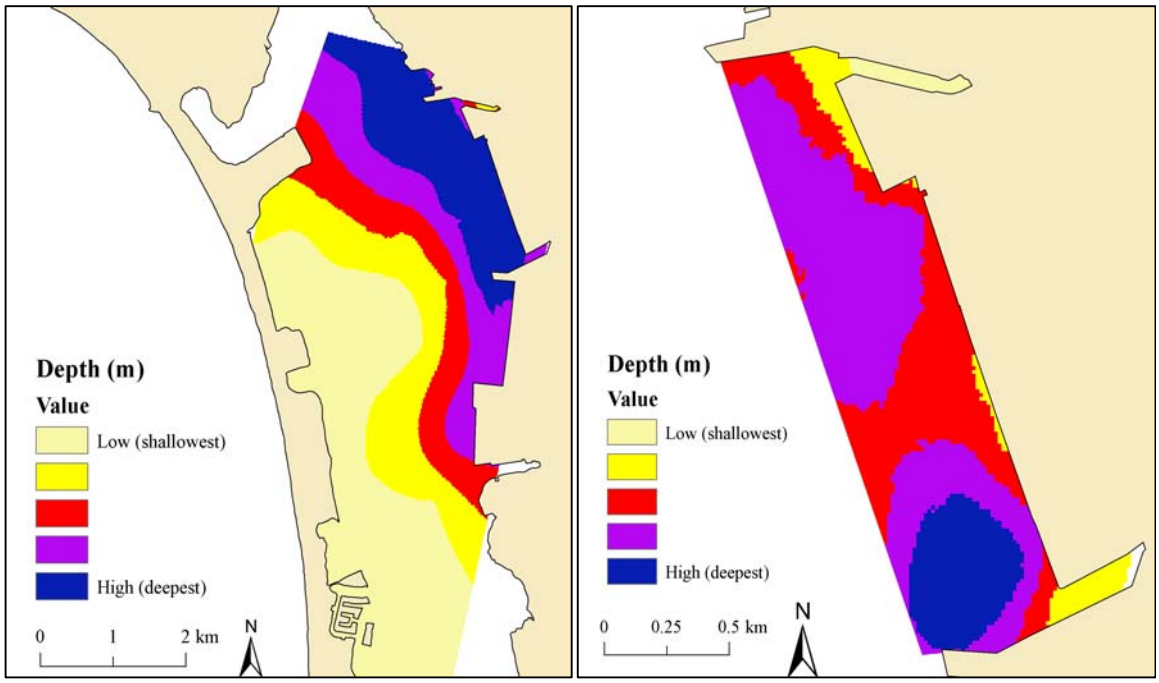

Figure 10-4. General spatial distribution of depth throughout the bay study area (left), and inner-harbor study area (right). The spatial distribution was estimated by interpolation of sample data (ordinary kriging), and is shown re-classified into a gradient of five value ranges (relative to each respective study area) using Natural Breaks classification.

GIS-Based Weights of Evidence Analysis

For each biological response variable (Table 10-1), categorical weights of evidence (WOE) analyses were performed to determine the spatial association between the biological response and each re-classified environmental variable raster. To distinguish between the effects of geographic sampling bias from evidence of a potential stressor-response association, two discrete training datasets representing low-quality (25th percentile) and high-quality (>75th percentile) sites were analyzed for each biological response variable. Figure 10-5 and Figure 10-6 illustrate examples of plotted WOE output, with an example of no significant spatial association delineated between biological response and an environmental variable (Figure 10-5), and an example of a significant potential stressor-response association (Figure 10-6). On the y-axis in Figures 5-6, the spatial association (studentized contrast) value indicates the relative increase (+) or decrease (-) in the odds of observing a training point in each value range of the stressor (x-axis) in comparison to chance (spatial association value value of zero). A spatial association (studentized contrast) value of 1.95 (absolute value) represents approximately 95% confidence for a significant spatial association (Robinson et al., 2004). For an additional description of WOE, see Agterberg (1992), Robinson et al. (2004) and Bonham Carter (1994).

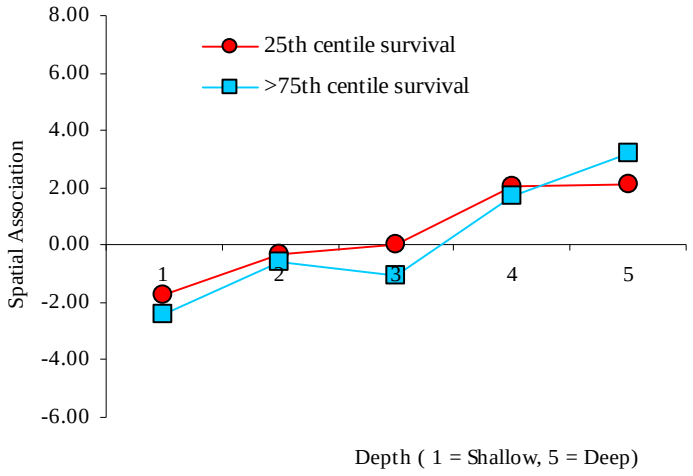

Figure 10-5. Example of WOE chart for amphipod toxicity which shows a similar spatial association (no evidence of stressor response) between low and high amphipod survival and site depth in the general harbor study area.

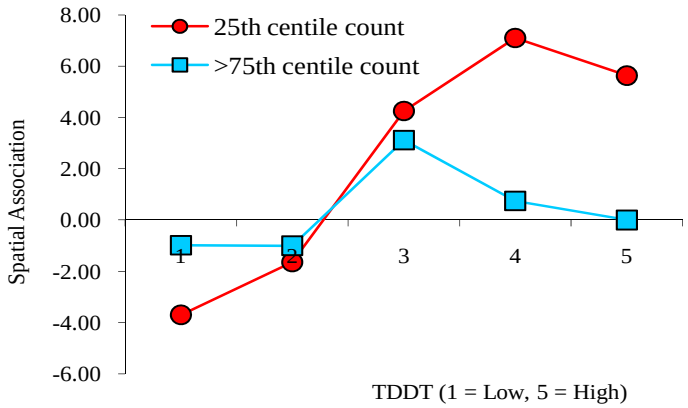

Figure 10-6. Example of WOE chart for total infauna abundance (count) which shows a comparatively distinct spatial association (significant stressor response) between benthic infauna total abundance and pesticides (TDDT) in the general harbor study area, with sites in the 25th centile associated with highest pesticide values.

Environmental variables were identified as potential stressors when the spatial association of low-quality sites (25th centile) were significantly different (magnitude of ~[1.95]) than that of higher quality sites for a particular level(s) of the environmental variable (ex. Figure 10-6). Relevance of the stressor-response association to ecological risk was also required in the stressor identification (i.e., the spatial association of low-quality sites with contaminants should be positive in order for the contaminant to be identified as a stressor). A methodology utilized for evaluating the data was simply subtracting the spatial association values for the high-quality sites (>75th centile, square symbols on charts) from the spatial association values of the low-quality sites (25th centile, circular symbols on charts). This approach provides a direct adjustment of spatial association for sampling bias, with similar trends between impacted and non-impacted sites resulting in trend close to zero, and potential stressor-response trends more clearly identified for trends that differed between low and high-quality sites (Figure 10-7). Environmental variable rasters identified as potential stressors as a result of WOE analysis were further re-classified to binary variables based on significant spatial associations to optimize their predictive strength. For example, in Figure 7 the pesticide variable was re-classified as: values 4-5= binary value of 1, values 1-3= binary value of 0). An additional WOE analysis was performed with the re-classified binary environmental variables to directly compute their spatial association with low-quality sites (results given in next section). Any binary variables with a spatial association (studentized contrast value) of $p>0.05$ were eliminated from further analysis. For each biological response variable, potential stressor variables were ranked by WOE spatial association. Prior to entering the potential stressor variables into a logistic regression model for each biological response, steps were taken to reduce effects of collinearity. First, potential stressors correlated at $R \geq 0.80$ (Spearman Rank correlation on raw sample data, Table 10-3) were identified, and the variable with the strongest spatial association to the biological response variable was selected for input in the proceeding logistic regression model. Next, prior to execution of the logistic regression models for each biological response variable, the environmental variables inputs for each model were analyzed for collinearity (SAS v. 9) by computing variance inflation factors (VIF) using the raw sample data. A VIF value of >2.5 was considered evidence of problematic collinearity, and if this was found with model input variables, the variable with the strongest correlation to others and the weakest WOE spatial association was removed from input to the model until all VIF values were below 2.5. These steps served as an approach to limit redundant information in the analysis, therefore improving statistical confidence and model interpretation.

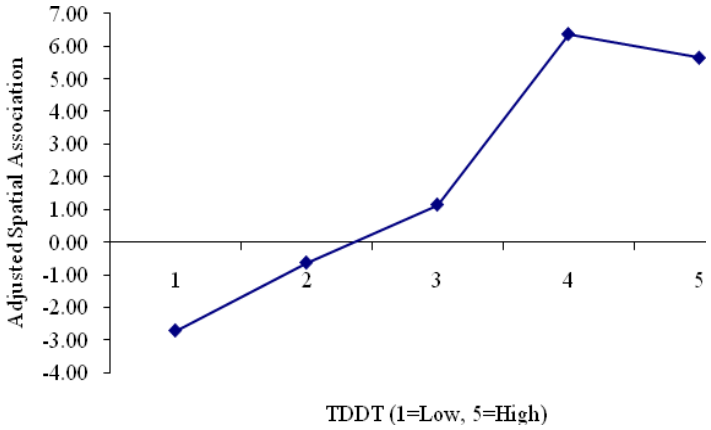

Figure 10-7. Example of adjusted WOE chart for total infauna abundance (count) based on subtraction of spatial association of high-quality sites from low-quality sites in Figure 6, more clearly demonstrating the spatial association between abundance and pesticides in the general harbor study area.

Weighted Logistic Regression (WLR)

Potential stressor variables selected and optimized (to binary) by the WOE data exploration process were entered as input to WLR models for each biological response. The WLR model estimates the influence of each potential stressor variable in the context of multiple (cumulative) potential stressors (as opposed to individual stressor-response associations in WOE data exploration). Regression parameter coefficients generated by the analysis were used as a relative ranking of stressor variable model influence, and corresponding raster maps delineating the relative probability of the occurrence of the biological response were generated for the study area based the spatial patterns of unique combinations of the potential stressor variables. Model fit was evaluated by computing the proportion of low-quality and high-quality sites (raw data) successfully predicted by the analysis (discussed and illustrated in the next section). Further description of WLR can be found in Agterberg (1992) and Raines et al. (2002). Advantages of this approach are the adjustment of probability computations to incorporate the study area extent (area) represented by each unique combination of stressors, as well as a lack of assumption of conditional independence of explanatory variables in the prediction of relative probabilities of the biological response.

10.4. Results and Discussion

General Harbor Study Area Results

As previously illustrated in Figures 5-7, WOE analysis quantitatively delineated spatial associations between biological response variables and environmental variables, allowing for identification of potential stressor-response relationships. Table 10-4 presents the individual variables identified and reclassified (to binary) by WOE analysis as potential stressors for the general harbor study area (narrowed down from the original 7 environmental variables given in Table 10-2). The term "potential stressor" in this study refers to an environmental variable that has a significant positive spatial association with low-quality sites (25th percentile of biological response variable, Table 10-1). For all biological response variables in the general harbor study area, pesticides (TDDT) had the comparatively highest spatial association with low-quality sites, with amphipod toxicity having the highest spatial association (Table 10-4). Amphipod toxicity had the highest number of potential stressor variables (4) identified by WOE, followed by species richness (3 potential stressors identified) and total abundance (2 potential stressors identified). All spatial associations were significant at $p<0.05$ (i.e., spatial association >1.95).

Table 10-4. General harbor study area potential stressor variables individually identified by WOE analysis.

Biological Response	Potential Stressor	Individual Spatial Association
Amphipod Toxicity	TDDT	8.35
	TOC	6.72
	Metals (Cu)	3.95
	% Fines	2.17
Total Abundance	TDDT	8.29
	PPAH	5.91
Species Richness	TDDT	7.76
	PPAH	3.93
	TOC	3.82

Evaluation of collinearity for the potential stressor variables identified for each biological response, as discussed in the previous section, was used to determine input into the corresponding logistic regression models. Variables removed from model input due to collinearity restrictions can still be considered potential stressors, however their individual contribution to the model would be redundant due to their correlation with one or more other model variables. Using this criteria, two potential stressor variables were entered into each logistic regression model. Potential stressor variables input into each model are given in Table 10-5, listed in the table by their relative model influence (parameter coefficient). Pesticides and priority pollutant PAHs predicted the spatial distribution of the 25th percentile of benthic infauna characterization variables, while pesticides and total organic carbon predicted the occurrence of sites in the 25th percentile of amphipod survival. All models, however, had much greater success predicting the absence of high-quality sites (80-85% predicted, Table 10-5) than the presence of low-quality sites (50-60% predicted, Table 10-5). This indicates that while the presence of elevated contaminants (and TOC in the case of amphipod toxicity) significantly increases the odds of observing a low-quality site and decrease the odds of observing a high-quality site, there are likely other environmental variables not evaluated in this study that are related to biological condition in the overall study area.

Table 10-5. General harbor study area WLR model explanatory variables for biological responses (listed in order by the magnitude of the parameter coefficient), with model fit represented as % sites successfully predicted. *= Model parameter coefficient significant at p<0.05 (Wald chi-square).

Benthic characterization	Model variables	25th centile predicted	>75th centile predicted
Species richness	TDDT*, PPAH[1] *	50%	85%
Total abundance	TDDT*, PPAH*	64%	80%
Toxicity	**Model variables**	**25th centile predicted**	**>75th centile predicted**
Amphipod *in situ* survival	TDDT*, TOC[2]*	60%	83%

[1] TOC is an additional potential stressor eliminated by collinearity restrictions, most correlated with PPAH.
[2] Metals (Cu) and % Fines were additional potential stressors eliminated by collinearity restrictions, most correlated with TOC.

The spatial pattern of model predictions provides additional information relevant to model stressor-response hypotheses and corresponding model fit. The highest relative probability of low-quality site occurrence predicted by the models for all three biological response variables was located primarily in the Chollas and Paleta Creek confluences (WLR mapping results, Figure 10-8 through Figure 10-10). These particular portions of the harbor study area dominated the model, as most sites with biological values in

the 25th centile were in fact located in these areas (apparent in Figure 10-2). The models provide a quantitative spatial representation of ecological risk throughout the harbor study area, and indicate that the Chollas and Paleta Creek areas, and to a lesser extent portions of the northern dock area and northern harbor study area have the highest relative risk to biological condition based on the potential stressors present at these locations. In a previous assessment of Chollas and Paleta Creeks from which sampling data was utilized (SCCRWP and SPAWAR Systems Center, 2005), pesticides, PAHs, and organic enrichment from both upstream inflow and shoreline activities were hypothesized as contributors to biological impairment for these areas using observations of co-occurrence and correlation analyses of multiple lines of evidence. The naval station dock area, which was heavily sampled for the 2008 SERDP project, was not indicated by the model to have a high potential for biological impairment (low-quality site occurrence based on the 25th percentile for the harbor study area). This finding was consistent with the relatively low impairment observed in the dock area. In fact, only three 2008 SERDP sample sites fell within the 25th percentile of harbor study area values for amphipod toxicity, and one 2008 SERDP site fell within the 25th percentile of harbor study area values for benthic infauna total abundance and species richness, respectively (Table 10-6). Out of the five 2008 SERDP sites that fell within the 25th percentile of a biological response variable, the WLR models predicted three of them based on total organic carbon (amphipod toxicity) and priority PAHs (total abundance). Other environmental conditions not evaluated in this study likely account for the biological impairment at the two sites not predicted by the modeling, and may play a role in conditions at the predicted sites as well. Overall, based on the spatial analysis results for the general harbor study area, the ecological risk of sediment contaminants in the naval station dock area sampled in the 2008 SERDP field program related to the biological responses evaluated in this study is relatively low, especially when compared with conditions at the confluences of Chollas Creek and Paleta Creek, where evidence of contaminant effects was significantly greater. As the spatial analysis was performed at the scale of the general harbor study area, model output and stressor-response hypotheses are relative to the particular study area. Environmental conditions predictive at a large study area scale may (or may not) change when analyzing stressor-response relationships within a smaller subset of the study area. Therefore, performing the spatial analysis at a smaller study area would provide additional information for an ecological risk assessment pertaining to specific geographic areas of interest.

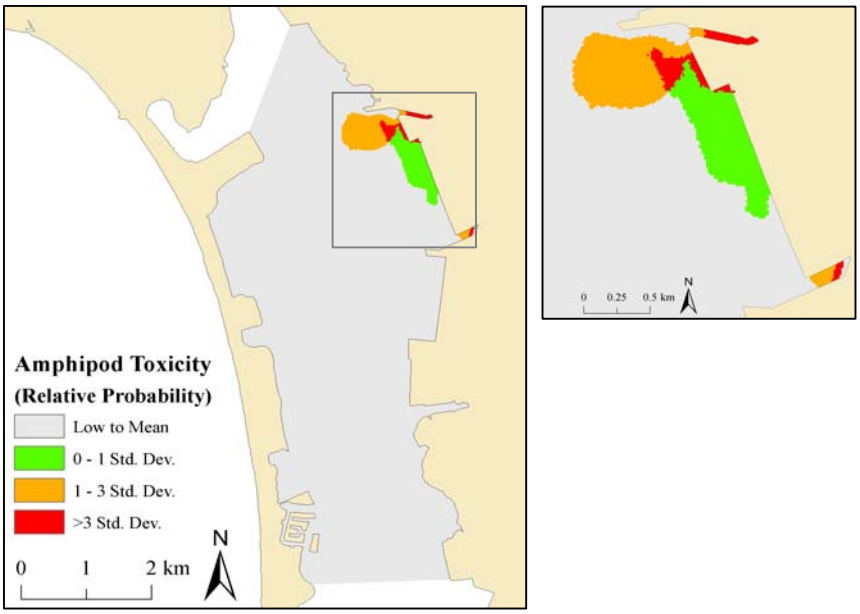

Figure 10-8. Relative probability maps generated by WLR for the harbor study area for the occurrence of sites in the 25th percentile of amphipod in situ survival. Relative impairment probability is given as standard deviations in order to compare between maps.

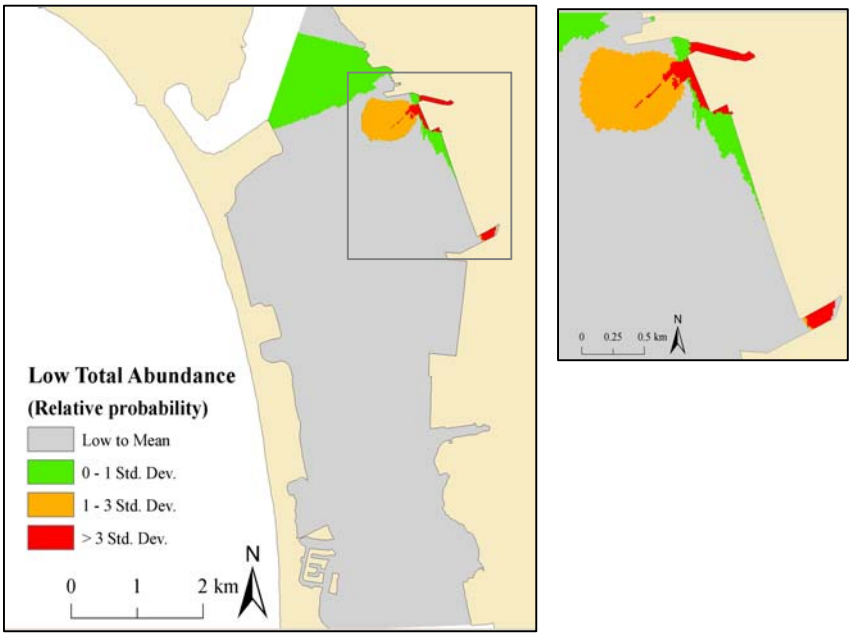

Figure 10-9. Relative probability maps generated by WLR for the harbor study area for the occurrence of sites in the 25th percentile of benthic infauna total abundance. Relative impairment probability is given as standard deviations in order to compare between maps.

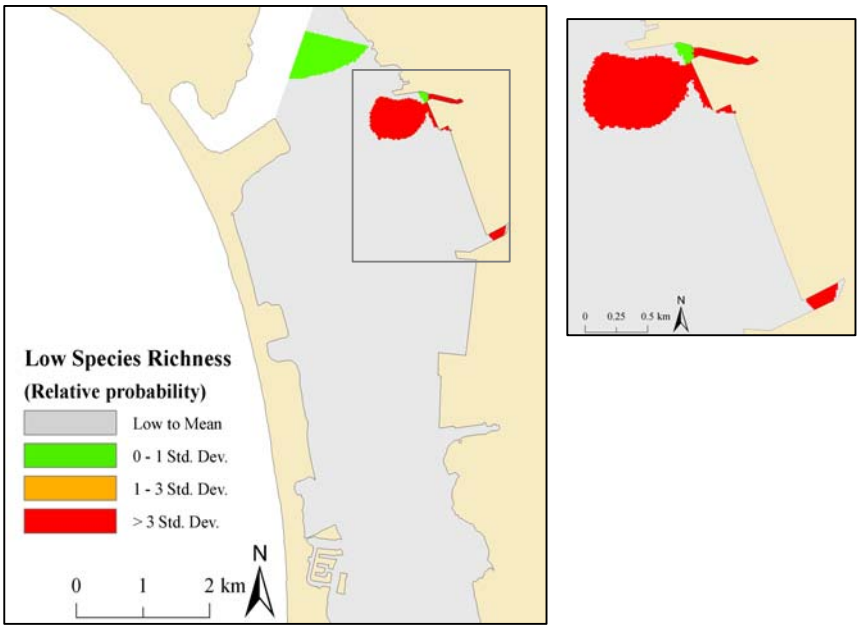

Figure 10-10. Relative probability maps generated by WLR for the harbor study area for the occurrence of sites in the 25th percentile of benthic infauna species richness. Relative impairment probability is given as standard deviations in order to compare between maps.

Table 10-6. WLR model results (general harbor study area) for 2008 SERDP sites, including the number of 2008 sites within the 25th percentile of biological response values, the percentage of those sites predicted by the WLR model, and the model stressor hypotheses for the site locations.

Biological Response	*N* in 25th percentile	Model Predicted	Stressors (Site ID)
Amphipod survival	3	2 (67%)	TOC (NS17, NS21), Other (NS28)
Total Abundance	1	1 (100%)	PPAH (NS7)
Species Richness	1	0 (0%)	Other (NS8)

Inner Harbor Study Area Results

As described in the *Methodology*, a WOE/WLR analysis was performed using a smaller subset of the harbor study area (Figure 10-3). The same criteria (thresholds) for low and high-quality biological condition used for the general harbor study area was also used for the inner harbor study area to keep definition of biological condition consistent for comparison between study areas. The specific environmental conditions within the inner harbor study area were represented by reclassifying interpolated environmental data within this area, so that environmental gradients were relative to the smaller study area. The individual variables identified by WOE analysis as potential stressors for the inner harbor study area are shown in Table 10-7 for the three biological response variables evaluated in this study. Pesticides (TDDT) had the highest individual spatial association with amphipod toxicity and species richness, while (low) total abundance was most associated with priority PAHs, followed closely by % fines and depth (Table 10-7). The collinearity evaluation, described earlier, resulted in only one potential stressor variable being eliminated from input into the logistic regression model (PPAH in species richness model, Table 10-8). Based on WLR model results, pesticides had the strongest relative influence on the modeled prediction of sites in the 25th percentile of biological response for amphipod survival and species richness, while depth had the strongest relative influence on the modeled prediction of sites in the 25th percentile for total abundance. Mapped WLR model results (Figure 10-11 through

Figure 10-13) display the modeled spatial prediction of low-quality sites for each biological response variable. As with the general harbor study area, most increased probability of biological impact occurs in the Chollas Creek and Paleta Creek portions of the study area, highlighting the significance of these sites as the primary locations of ecological risk relative to the rest of the study area. The prediction rates for low-quality sites in the inner harbor study area models were improved compared with the general harbor study area prediction rates (Table 10-5).

Table 10-7. Inner harbor study area potential stressor variables individually identified by WOE analysis.

Biological Response	Potential Stressor	Individual Spatial Association
Amphipod Toxicity	TDDT	4.48
	TOC	3.07
Total Abundance	PPAH	4.30
	% Fines	4.27
	Depth	4.13
	TDDT	3.73
Species Richness	TDDT	3.66
	Depth	2.25
	PPAH	2.12

Table 10-8. Inner harbor study area WLR model explanatory variables for biological responses (listed in order by the magnitude of the parameter coefficient), with model fit represented as % sites successfully predicted.

Benthic characterization	Model variables	25th centile predicted	>75th centile predicted
Species richness	TDDT*[1], Depth	53%	92%
Total abundance	Depth*, PPAH, TDDT, %Fines	82%	75%
Toxicity	**Model variables**	**25th centile predicted**	**>75th centile predicted**
Amphipod *in situ* survival	TDDT*, TOC	71%	91%

[1] *PPAH is an additional potential stressor eliminated by collinearity restrictions, most correlated with TDDT.*

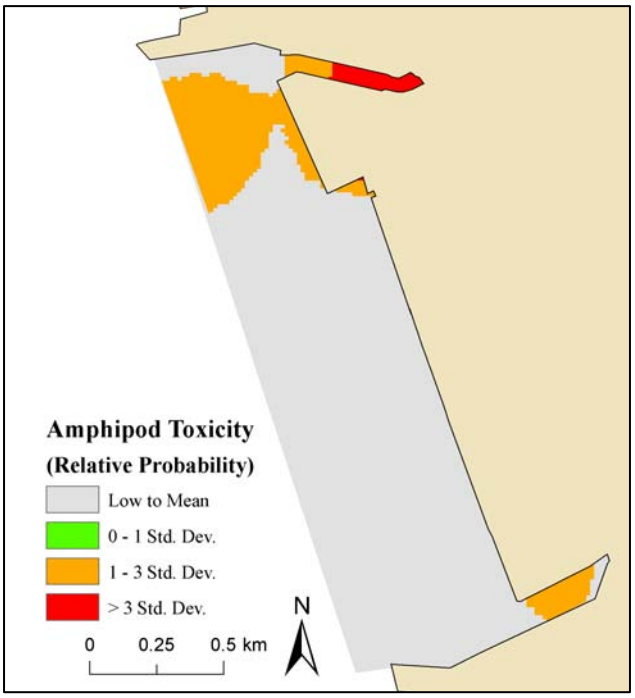

Figure 10-11. Relative probability maps generated by WLR for the harbor study area for the occurrence of sites in the 25th percentile of amphipod in situ survival. Relative impairment probability is given as standard deviations in order to compare between maps.

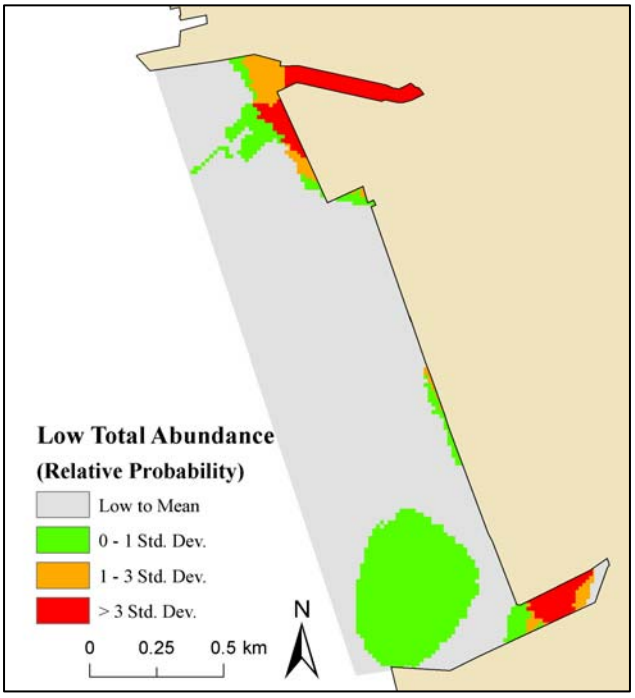

Figure 10-12. Relative probability maps generated by WLR for the harbor study area for the occurrence of sites in the 25th percentile of benthic infauna total abundance. Relative impairment probability is given as standard deviations in order to compare between maps.

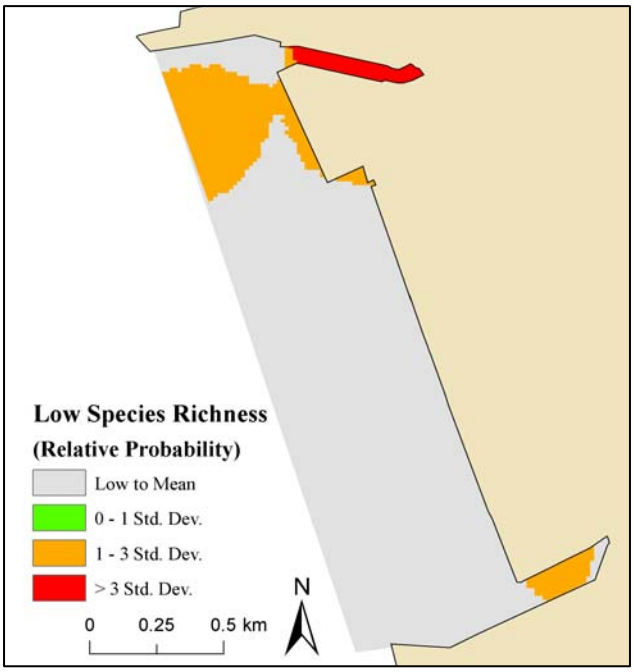

Figure 10-13. Relative probability maps generated by WLR for the harbor study area for the occurrence of sites in the 25th percentile of amphipod in situ survival. Relative impairment probability is given as standard deviations in order to compare between maps.

The potential stressor variables and magnitude of relative model influence between the general harbor study area and inner harbor study area were further compared (Figure 10-14 through Figure 10-16). For amphipod toxicity, the same two variables (pesticides and total organic carbon) were identified as potential stressor variables, with pesticides having the strongest relative influence on the predicted spatial distribution of amphipod toxicity (Figure 10-14). Model influence for these variables was stronger at the general harbor study area scale compared with the smaller inner harbor study area, but the trends were quite similar. The delineation of this stressor-response relationship at both the general harbor study area scale and the smaller inner harbor study area scale strengthens the evidence that pesticides and total organic carbon are factors relevant to ecological risk (for areas of elevated risk as delineated in Figure 10-11). In contrast, the inner harbor model for total abundance was quite different from the general harbor study area model, with depth having the greatest relative influence in the inner harbor study area compared with pesticides and priority PAHs in the general harbor study area (Figure 10-15). This indicates that while contaminant levels (pesticide and PAHs) are predictive of lower total abundance at the scale of the larger harbor study area, these contaminants are significantly less predictive within the inner harbor study area itself when compared with depth (and other environmental conditions that may associated with depth not accounted for in this study). Finally, for species richness, pesticides (TDDT) were the most influential model variable for both study areas (to a lesser extent in the inner harbor study area, Figure 10-16). Priority PAHs were predictive at the general harbor study area scale, but collinearity restrictions prohibited its inclusion in the inner harbor model. However, this variable was identified as a potential stressor for species richness in WOE analysis. The differences in relative influence between the general harbor study area and smaller inner harbor study area demonstrate the importance of assessment scale, as stressor-response hypotheses for site locations may differ based on the data extent. One spatial extent is not necessarily preferred over another, as both provide hypotheses directly relevant to each respective study area, including the stressor-response relationships acting upon the particular assessment scale.

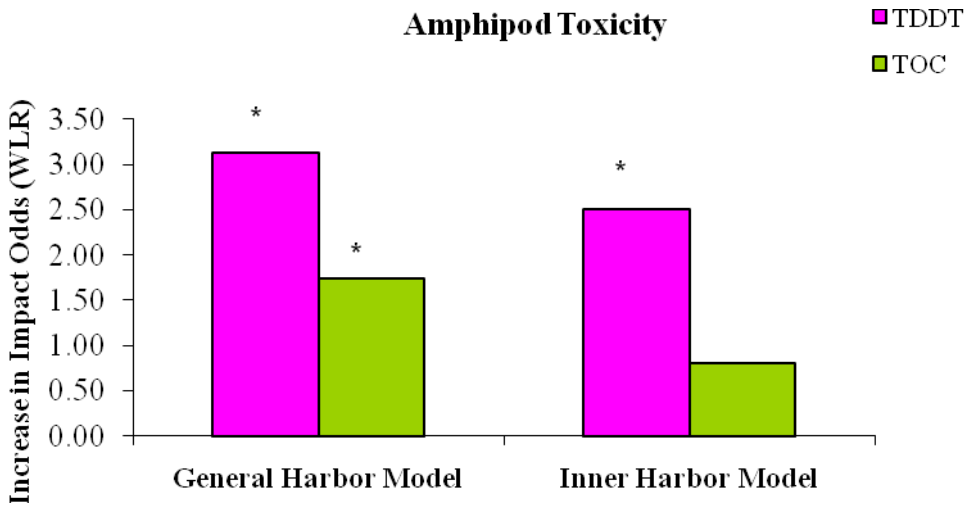

Figure 10-14. Comparison of WLR model influence (increase in relative odds of impact, i.e. observing a site in the 25th percentile) of identified potential stressors for amphipod survival between the general harbor study area model, and the smaller inner harbor stud y area model, and the smaller inner harbor study area model. * = coefficient significant at p<0.05, Wald chi-square.

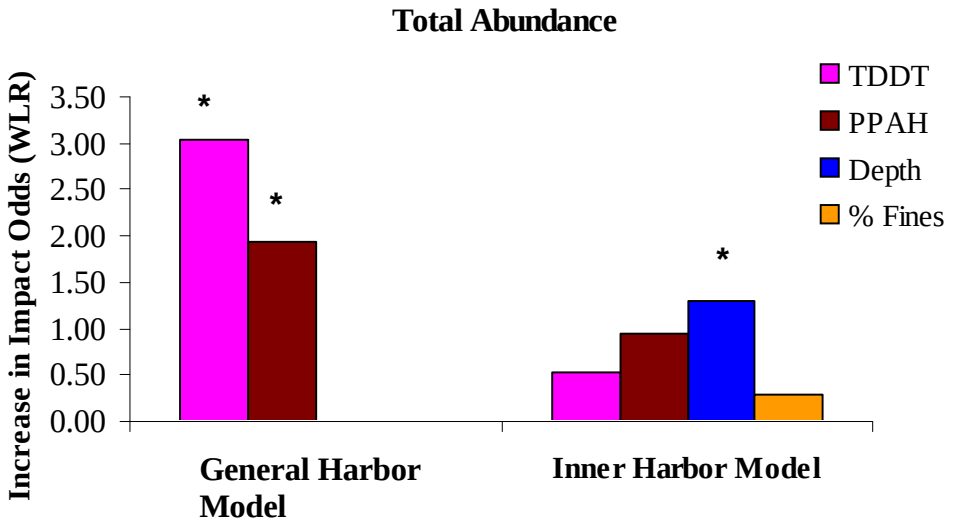

Figure 10-15. Comparison of WLR model influence (increase in relative odds of impact, i.e. observing a site in the 25th percentile) of identified potential stressors for benthic infauna total abundance between the general harbor study area model, and the smaller inn er harbor study area model. * = coefficient significant at p<0.05, Wald chi-square.

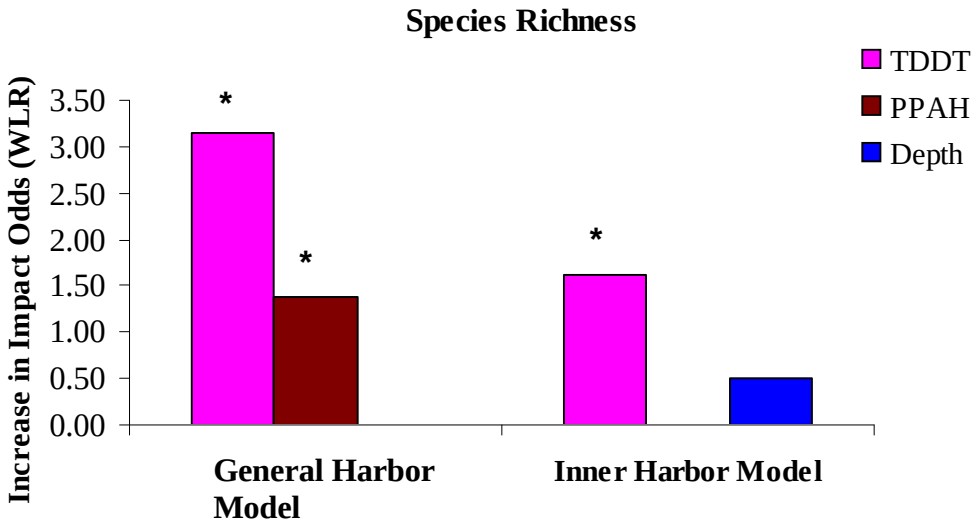

Figure 10-16. Comparison of WLR model influence (increase in relative odds of impact, i.e. observing a site in the 25th percentile) of identified potential stressors for benthic infauna species richness between the general harbor study area model, and the smaller inner harbor study area model. * = coefficient significant at p<0.05, Wald chi-square.

The inner harbor study area model did not predict biological impairment (low-quality site occurrence) for any sites within the naval station dock area sampled in the 2008 SERDP field program (Figure 9-3Figure 10-11 through Figure 10-13). Stressor-response associations delineated for the inner harbor study area were mainly limited to the Chollas and Paleta Creek areas. Ecological risk was delineated as low for most of the portion of the inner harbor study area sampled in the 2008 SERDP field program. Therefore, the ecological risk from sediment contamination in the naval station dock area (based on the variables used in this study) is estimated to be relatively weak, with biological condition in this area likely dependent on other environmental factors such as water chemistry or natural habitat characteristics not evaluated in this study. The % Fines variable used in this study was the only physical habitat characteristic consistent between the various monitoring programs, however when examining the 2008 SERDP data which sampled a number of physical sediment characteristics this variable was highly correlated (Spearman correlation >0.90) to % Sand, % Silt, and % Clay. Therefore, it is likely the % Fines variable is already generally representative of these particular physical sediment characteristics. The influence of depth delineated for benthic infauna responses for the inner harbor model may indicate natural influences on site biological condition not sampled in this study but related to depth, for example primary productivity.

Uncertainty

Overall, the general lack of highly impacted sites skewed the definition of "low-quality" to include sites with relatively low disturbance. For example, the average survival for amphipod toxicity sites considered "low-quality" (the 25th percentile of survival results) was 73%, while average survival for "high-quality" sites (>75th percentile) was 89%. This factor should be considered in interpretation of analysis results, as "ecological risk" modeled in this study does not necessarily mean an extremely large impact. Deviance from a reference biological condition may be an improved approach to construct measures of biological impact in future studies. However, for areas where biological condition is generally moderate to high, the targeting of especially high-impact sites may yield an inadequate sample size for statistical computations to be performed. The use of the relative measure of biological condition (percentiles) in this study provided an adequate and similar sample size for low and high-quality sites. Other uncertainty in the analysis results includes the linking of monitoring datasets sampled in different time periods. As

discussed earlier, the use of other monitoring datasets to supplement the 2008 SERDP field data also reduced uncertainty associated with data interpolation for developing estimations of the distribution of environmental variables across the study area. Biological and environmental variables selected for this study were based on consistent representation between monitoring programs, as well as their representation of various biological conditions and general environmental factors of interest. However, the use of cumulative measures of sediment contaminants, while useful in identifying general exposure and effects of contaminants, may fail to capture specific impacts of single compounds which are not highly individually related to a contaminant category.

10.5. Conclusions

Stressor-response hypotheses generated by the spatial analyses can provide insight into the process(es) influencing local recovery or degradation, and provide guidance for corresponding remedial strategies if deemed necessary. Despite uncertainties to the study as discussed above, overall the application of a WOE/WLR-based ecological assessment to benthic survey and *in situ* toxicity field data for the Naval Station study area was successfully technically applied to SERDP project data for the San Diego harbor study area, and effectively delineated screening-level stressor hypotheses for use in site management. The study results indicated that ecological risk and associated remediation strategies in the harbor would be best focused on the Chollas and Paleta Creek areas, as the dock area of the inner harbor sampled during the 2008 SERDP study had comparatively lower levels of risk. For areas with predicted ecological risk, pesticide exposure (represented as cumulative pesticide exposure) generally provided the greatest increase in ecological risk, pointing to this stressor source as remediation priority. This study showed potential for the application of this type of spatial analysis approach to other harbor-based study areas, particularly those with greater data variability (i.e., more abundant and severe instances of biological impact) and a sampling design limiting geographic sampling bias further.

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APPENDIX A: SCIENTIFIC/TECHNICAL PUBLICATIONS

1. Articles in peer-reviewed journals

Burton Jr, GA, Rosen G, Chadwick B, Greenberg MS, Taulbee K, Lotufo GR, Reible DD (in prep). A Sediment Ecotoxicity Assessment Platform for *In Situ* Measures of Chemistry, Bioaccumulation and Toxicity. A Sediment Ecotoxicity Assessment Platform for *In Situ* Measures of Chemistry, Bioaccumulation and Toxicity. Part 1: System Description and Proof of Concept.

Rosen G, Burton Jr, GA, Chadwick B, Greenberg MS, Taulbee K, Lotufo GR, Reible DD (in prep). A Sediment Ecotoxicity Assessment Platform for *In Situ* Measures of Chemistry, Bioaccumulation and Toxicity. Part 2: Multiple Lines of Evidence at a Contaminated Sediment Site.

Rosen G, Miller K, in press. A post exposure feeding assay using the marine polychaete *Neanthes arenaceodentata* suitable for laboratory and in situ exposures. *Environmental Toxicology and Chemistry.*

Rosen G, Lotufo GR, 2010. Fate and effects of Composition B in multispecies marine exposures. *Environmental Toxicology and Chemistry* 29:1330-1337 (leveraged paper).

2. Technical Reports

Rosen G, Chadwick DB, Poucher SL, Greenberg MS, Burton GA. 2009. *In Situ* Estuarine and Marine Toxicity Testing: A Review, Including Recommendations for Future Use in Ecological Risk Assessment. Space and Naval Warfare Systems Center Pacific (SSC Pac) Technical Report 1986. September 2009. 73pp.

3. Conference/Symposium abstracts

Rosen G, Chadwick, DB, Greenberg MS, Burton, GA, Jr., 2010. Evaluation of an Integrated Exposure and Effects Assessment Approach Involving *In Situ* and Laboratory Tools Along Three Contamination Gradients. Poster presentation, 31st Annual Meeting of SETAC, Portland, OR, Novebmer 7-11, 2010.

Rosen G, Chadwick, DB, Greenberg MS, Burton, GA, Jr., 2009. Development of a novel in situ based monitoring approach for contaminated sediment assessment. Oral presentation, 5th International Conference on Remediation of Contaminated Sediments, Jacksonville, FL, Feb 2-5, 2009.

Rosen G, Chadwick, DB, Greenberg MS, Burton, GA, Jr., 2009. Development of a novel in situ based monitoring approach for contaminated sediment assessment. Oral presentation, Navy and Marine Corps Cleanup Conference, Oxnard, CA, February 24, 2009.

Rosen G, Chadwick DB, Greenberg MS, Burton, GA, Jr., 2008. Linkage of Exposure with Biological Effects Using In Situ-Based Monitoring Tools in Marine and Estuarine Systems, Oral presentation, 29th Annual Meeting of SETAC, Tampa, FL, November 19, 2008.

4. Patents

US Patent Pending- Navy Case No. 99948: In Situ Sediment Ecotoxicity Assessment System.